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1. Your reference GBP87988

2. Patent application number (The Patent Office will fill in this part) 0313737.9

13 JUN 2003

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Isis Innovation Limited,
Ewert House
Ewert Place
Summertown
Oxford
Oxfordshire OX2 7SG
United Kingdom

Patents ADP number (if you know it) 39985640003.

If the applicant is a corporate body, give the country/state of its incorporation: United Kingdom

4. Title of the invention: Improved Vaccines.

5. Name of your agent (if you have one)
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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| Country | Priority application No (if you know it) | Date of filing (day / month / year) |
|---|---|---|

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| Number of earlier application | Date of filing (day / month / year) |
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# IMPROVED VACCINES

The present invention provides novel methods of vaccination.

Vaccination is a useful tool for combating and preventing disease caused by a number of agents. Such agents may be exogenous, such as viruses, bacteria or parasites. Alternatively, disease-causing agents may be endogenous, such as tumours. Immune responses induced by vaccination may be divided into humoral or antibody responses, and cellular immune responses, such as those mediated by T lymphocytes. A large number of vaccines act by generating protective levels of antibodies.

Antibody-inducing vaccines frequently employ either an entire microorganism, that is often attenuated or heat-killed, or a sub-unit component of the microorganism with an adjuvant. Favoured adjuvants for inducing strong antibody responses include Alum, NP59, CpGs, AS02 and various emulsions.

However, it has recently become clear that cellular immune responses may also be of some use on their own. Various means have been used to induce strong protective cellular immune responses by vaccination.

Known means of generating strong cellular immune responses include DNA vaccination, immunisation with viral vectors or protein particles, the use of recombinant bacteria such as BCG, and heterologous prime-boost immunisation. In fact, heterologous prime-boost immunisation approaches have been found to induce particularly strong effector T cell responses in animals and humans. Such regimes may comprise priming with DNA followed by boosting with recombinant modified virus Ankara (MVA), or priming with recombinant fowl pox and then boosting with recombinant MVA.

In order to combat these more effectively, it is desirable to induce stronger immune responses. However, vaccination methods that generate high level antibody

responses differ significantly from those that engender strong cell-mediated or T cell responses. For example, Alum is a useful adjuvant for inducing antibodies but generates weak or negligible $CD8^+$ T cell responses. In contrast, heterologous prime-boost immunisation methods have induced strong T cell responses in humans, but only minimal antibody responses. However, immune protection against many diseases can be mediated by either T cells or antibodies at sufficient levels, and optimal protection may be achieved by inducing strong responses of both types. For example, in malaria, it is thought that a pre-erythrocytic vaccine capable of inducing of both high level anti-sporozoite antibodies as well as a strong T cell response against the liver stage parasite would be an ideal method of preventing or treating the disease. However, no vaccination approach currently exists that allows strong responses of each type to be generated.

This problem exists not only in malaria, but in a large number of other diseases. Accordingly, there is a need in the art for a method of vaccination that can not only induce a high level antibody response, but also a strong cellular or T cell mediated response. The present invention sets out to overcome this need by providing novel vaccination methods capable of inducing both strong antibody and T cell responses.

Surprisingly, the present inventors have found that co-administration of an antigen with a viral vector induces both strong antibody and cell-mediated immune responses in a target mammal.

The antigen and the viral vector may be administered together or separately, and may be administered at the same time or over a period of time, preferably on the same day, particularly within 2 to 3 hours, and more preferably substantially together.

Administration of the antigen with the viral vector surprisingly stimulates both a humoral and an antibody response to the antigen, thereby providing an immune response on both levels.

The nature of the viral vector is not critical to the present invention. In general the vector should be able to stimulate a T cell response. Suitable examples of viruses are provided below. It is preferred that the viral vector is incapable of causing a serious

infection in the patient, and it is generally preferred that the virus is incapacitated, such as by heat treatment or attenuation. Empty capsids may be employed.

The present invention further provides vaccines comprising both the antigen and viral vector of the invention, as well as kits comprising preparations thereof.

Preferably, the antibody levels induced in the target mammal are greater in the vaccination method according to the present invention than those seen on administration of the antigen alone. Furthermore, it is also preferred that the T cell response induced as a result of the vaccination method according to the present invention is not less than an order of magnitude less than that induced by administration of the viral vector alone. Preferably, the T cell response induced by the present invention is not decreased, and more preferably is increased, compared to the T cell response elicited by the viral vector alone. Most preferably, both the levels of antibody and the T cell response induced by the present invention are greater than that achieved by administration of the antigen or the viral vector alone.

Preferably the antigen is proteinaceous. Alternatively, it is also preferred that the antigen is a peptide. Furthermore, it is preferred that the viral vector is attenuated, heat-killed or unable to replicate. Whilst it is envisaged that the viral vector is derived from any virus known in the art that is suitable for use therefor, it is particularly preferred that the viral vector is selected from the group consisting of retroviruses, adenoviruses and adeno-associated viruses. More preferably, the viral vector is derived from the herpes viridae family, preferably the varicella viruses, or from the pox viridae family, preferably MVA or fowl pox.

The antigen and the viral vector are preferably administered within several days of each other but more preferably within 3 hours and most preferably within one hour of each other. Alternatively, the antigen and the viral vector may be administered at the same time, either separately, or more preferably, as a mixture.

Administration of the antigen and the viral vector, whether independently or as a mixture, may be orally or transdermally, but most preferably parenterally. The site of

parenteral administration may be intravenously, intramuscularly, subcutaneously, or intradermally, or by any other means known to the skilled person.

The vector may, optionally, comprise a nucleic acid encoding a protein or peptide. The expressed product of this nucleic acid is preferably an antigen. This expressed antigen may be homologous to the antigen administered with the viral vector, but may also be heterologous. Preferably, the expressed protein or peptide is recombinant.

The antigen may be derived from a virus, such as HIV or the Hepatitis B virus, preferably the epitope is derived from a coat or backbone protein. In a preferred embodiment, the antigen comprises the Hepatitis B surface Antigen (HbsAg). However, it is also preferred that the antigen is derived from a parasite, such as a malarial parasite from the *Plasmodium* family, a bacteria, such as *M. tuberculosis*, or may even be endogenously derived, for instance a tumour antigen.

The vector may comprise no antigen-encoding nucleic acid. Accordingly, it is preferred that the vector comprises a nucleic acid encoding a marker such as Lac Z, but no antigenic protein or peptide. Preferably, the vector is an empty vector.

In combating HIV, neutralization or opsonisation of HIV-1 by antibodies combined with the action of CTL suppression of viral replication and induction of cell death of virally infected cells, may have a multiplicative effect on $R_o$, the mean number of cells that are infected by a single infected cell. By reducing $R_o$, the spread of virus and, therefore, viral load, may be limited. This may allow the immune system to focus on rapidly controlling initial infection. Limiting the amount of viral replication may also reduce the virus' opportunity to mutate following immune pressure of specific epitopes which may, therefore, limit viral escape.

The method of vaccination according to the present invention may simply comprise administration of the antigen and the viral vector. Alternatively, the method may comprise administration of other elements such as adjuvants, such as Alum, or other vaccines. The method may also be part of a more complex vaccination regimen,

for instance that comprising a homologous, but preferably a heterologous, prime-boost vaccination regimen.

Therefore, although the present invention may be used in isolation, it may also be combined with other vaccination regimens for combating or preventing the same disease. Alternatively, it may be part of a regimen for combating or preventing more than one disease. Accordingly, the present invention may be used in a regimen that elicits an immune response to more than one antigen. For instance, the viral vector may comprise a nucleic acid that encodes a protein or peptide derived from HIV, whilst the antigen administered according to the present invention is derived from *M. tuberculosis*. Therefore, in another embodiment of the invention, the viral vector may encode a protein or peptide that is derived from a different disease-causing agent than the antigen co-temporaneously administered with the vector.

The T cell response induced is preferably an effector T cell response. Preferably, the response comprises a $CD4^+$ T Helper cell response, or a $CD8^+$ Cytotoxic T Lymphocytes (CTL) response, and most preferably both, such as a CTL response mediated by T Helper cells, for instance.

The invention is now described by way of illustration only in the following examples in which the antigen is HBsAg. An Elisa assay was used to measure the anti-HBsAg antibody response. IFN-γ Elispot assays were used to measure T cell responses to whole antigen (HBsAg) or peptide (CD8+ epitope IPQSLDSWWTSL). Statististical analysis was provided using the SPSS 11.0 program to perform Mann-Witney U tests (non-parametric test, 2 independent samples). Exact significance [2* 1-tailed].

A number of commercially available vaccines are available for Hepatitis B that induce protective levels of antibodies. However, not all patients successfully sero-convert to protective levels (Alper FE *et al* 1995 exp clin immunogenet 12:171-81). These vaccines induce high levels of anti-HBsAg antibody but a minimal specific effector T-cell response as measured, for example, by the widely used interferon-gamma ELISPOT assay. As discussed above, one of the current challenges facing vaccinology is the identification of means to induce strong humoral and cellular

response concurrently. Both responses may be of value in preventing and treating a number of diseases including hepatitis B virus infection. Inducing both responses may provide protection to the percentage of the population who fail to sero-convert to the standard alum-adjuvanted commercially available vaccine (for example, the Engerix B product), and in the treatment of persistent hepatitis B virus infection.

DNA and MVA has been used for a number of years in laboratory animals and in many human clinical trials as a vector for the antigen or antogens of interest (Schneider *et al* 1999 imm.Rev, Hanke, Vaccine. 2002 May 6: 20(15): 199-8,. Matteo 1999 J immunol 163:4058) in order to induce strong effector CD4+ and CD8+ T cell responses.

# Experiments

## Experiment 1

Aim:

Induction of a strong humoral response to HBsAg while still maintaining a strong cellular response by combining a vaccine to induce protective antibody levels (Engerix-B contains HBsAg and PreS regions of HBV adsorbed to Alum) with a T-cell inducing regime (DNA prime and MVA boost, both containing HBsAg and PreS regions of HBV).

### 1.1 Antibody Responses

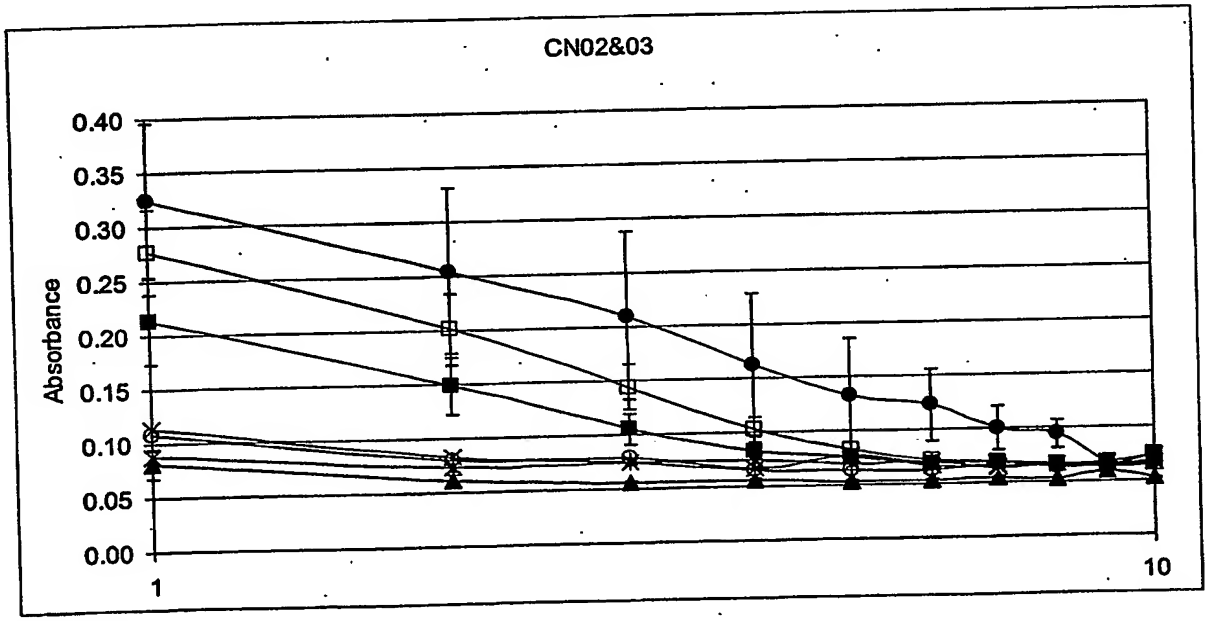


**Figure 1**

n=3-6 +/- SEM

PRIME
1. ▲ DNA.HBs i.m.
2. ○ Nil
3. ● DNA.HBs i.m.
4. □ DNA.HBs i.m.
5. ■ DNA.HBs i.m.
6. × DNA.HBs i.m.
7. * Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

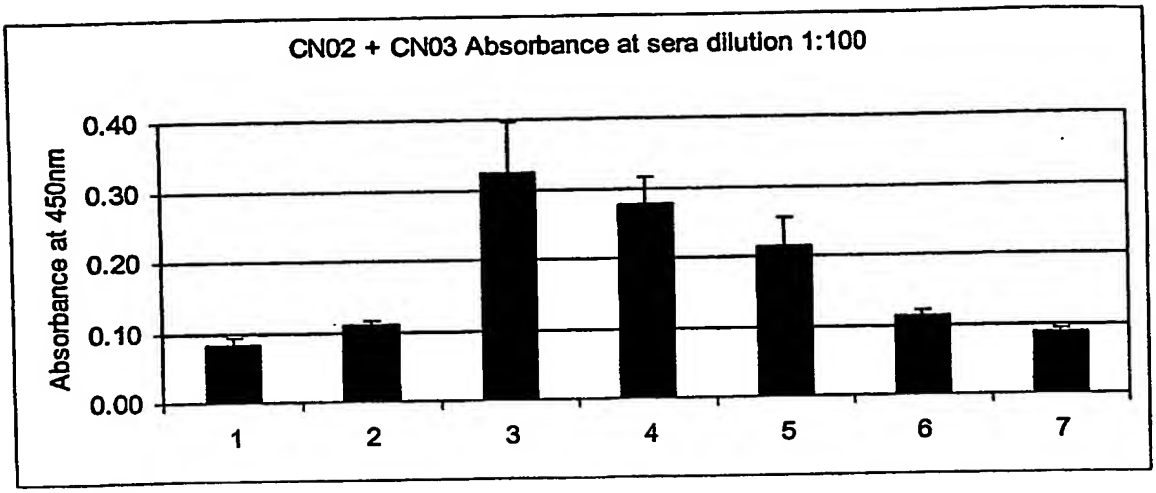


**Figure 2**

n=3-6 +/- SEM

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

**MVA.HBs Adjuvants Engerix-B**

DNA.HBs prime followed by MVA.HBs mixed with Engerix B and administered s.c. produced the strongest antibody response. The next best immunisation regime was DNA.HBs prime followed by MVA.HBs i.v. and Engerix B s.c.

This indicated that, surprisingly, MVA.HBs when mixed with Engerix-B (group 3) was further adjuvanting the HBsAg in the Engerix-B vaccine leading to a stronger antibody response than was observed when the Engerix-B and MVA.HBs were administered separately (group 4). This is further supported by DNA.HBs priming followed by Engerix-B boosting (group 5) which gave lower responses than when MVA.HBs was included in the boost (group 3).

**DNA.HBs priming increases antibody responses to Engerix-B**

DNA.HBs however, was in part responsible for the increase in antibody responses. One shot of Engerix B at week 2 (no immunization at prime) gave significantly lower antibody responses than DNA.HBs priming followed by boosting with Engerix-B with or without MVA.HBs (s.c. or i.v) ($p=0.026$, 0.002, 0.002 respectively). DNA.HBs priming followed by MVA.HBs boosting gave the lowest antibody response, not significantly different from unimmunised animals ($p=0.931$).

MVA.HBs boosts antibody response when administered at the same time as Engerix B either at the same or different sites.

DNA.HBs priming increases the antibody response to a single shot of Engerix B given 2 weeks following priming.

## 1.2 T-Cell Responses

### 1.2.1 Peptide stimulated splenocytes

DNA.HBs prime and MVA.HBs boost gave the strongest T cell responses but not significantly different to DNA.HBs priming followed by boosting with Engerix-B and MVA.HBs(s.c. or i.v) (p=0.343 both s.c. and i.v.).

DNA.HBs priming followed by boosting with Engerix-B and MVA.HBs i.v was significantly better than DNA,HBs priming followed by Engerix-B boosting (p=0.029). This suggests that MVA.HBs is responsible for inducing high levels of specific T-cells and that this response is not significantly reduced when combined with Engerix-B.

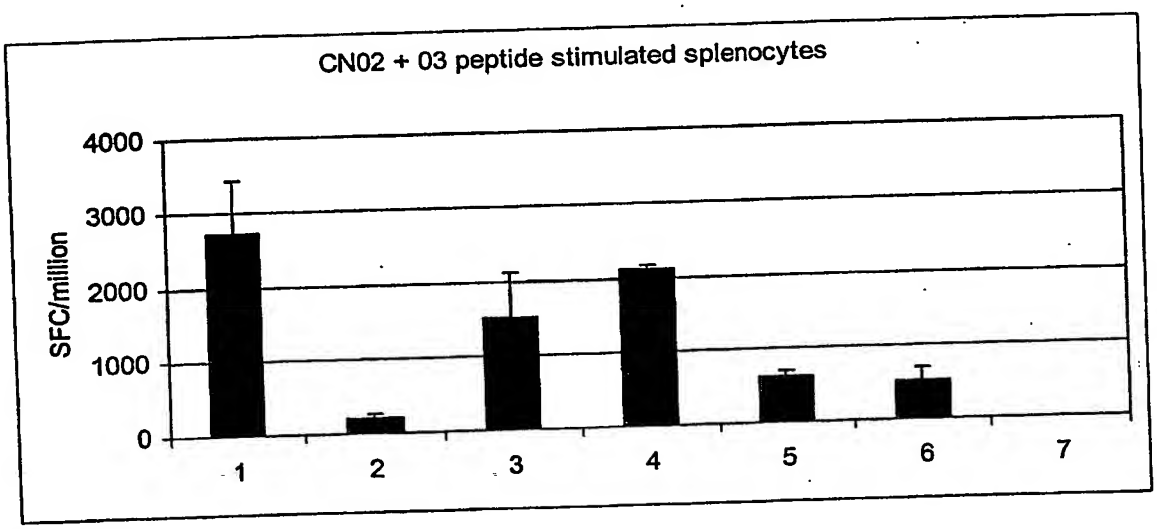


**Figure 3**

n=3-6 +/- SEM

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

## 1.2.2 HBsAg Stimulated Splenocytes

A similar patter to peptide stimulated splenocytes is seen upon HBsAg stimulation, although much lower overall response levels, probably because processing and presentation of the entire protein is incomplete; no processing is required for peptide presentation.

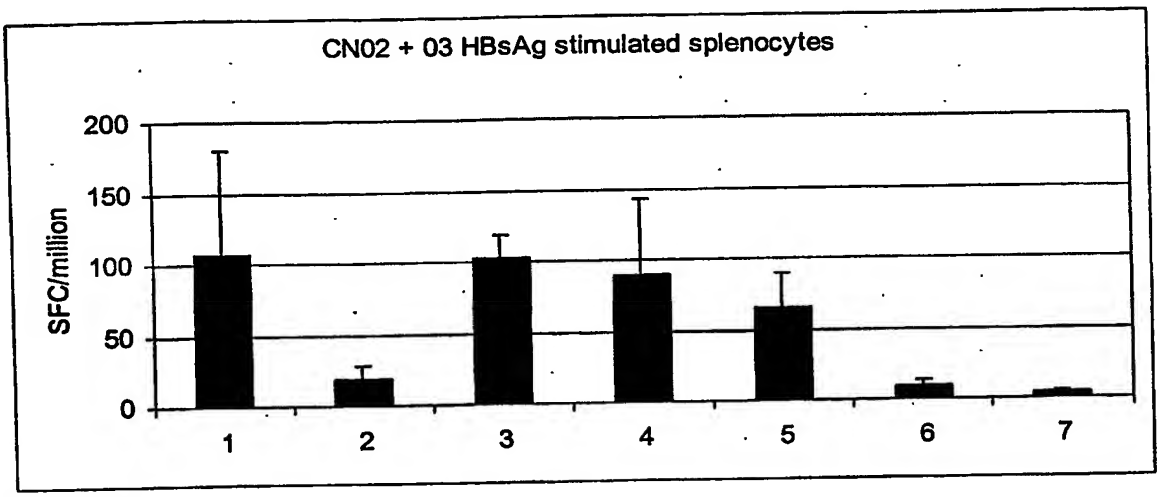


**Figure 4**

n=3-6 +/- SEM

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

### 1.2.3 Peptide Stimulated Lymph Nodes

Again, a similar pattern to peptide stimulated splenocytes. Cells from cervical and axial lymph nodes for each group were pooled. The strongest response was seen in DNA.HBS primed and MVA.HBs i.v. and Engerix-B boosted animals.

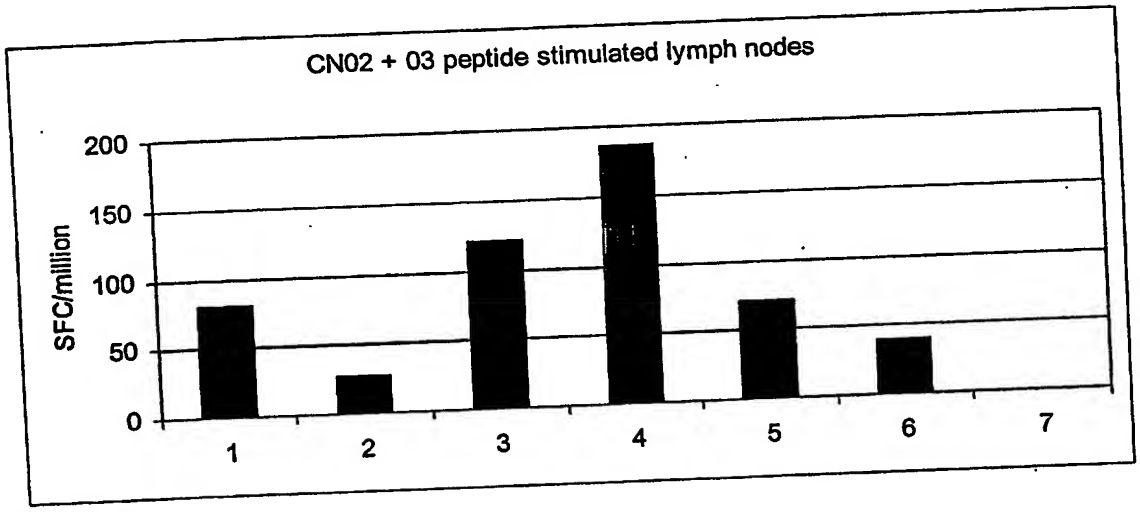


**Figure 5**

n= cells from 3-6 animals pooled

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

## 1.2.4 HBsAg Stimulated Lymph Nodes

Responses to whole antigen was well below 50 SFC/million except for animals primed with DNA.HBS and boosted s.c. with MVA.HBs and Engerix-B where cell numbers were 212 SFC/million.

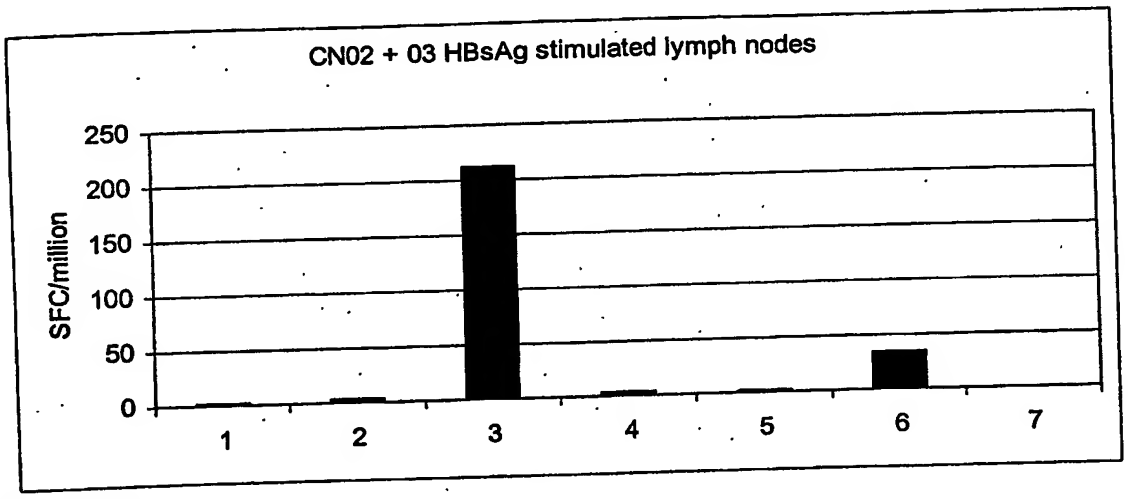


**Figure 6**

n= cells from 3-6 animals pooled

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

# Experiment 2

To further increase Ab levels by including Engerix-B in the prime and still maintain T-cell responses. To establish whether the enhancement still apply when the route of MVA administration is changed to intradermal (id) applies and to bring it closer to the subcutaneous (sc) immunisation site of Engerix-B, so that both immunizations share the same draining lymph node. To establish whether an MVA vector not encoding an antigen (MVA.LacZ) boosts responses to HBsAg

## 2.1 Antibody responses

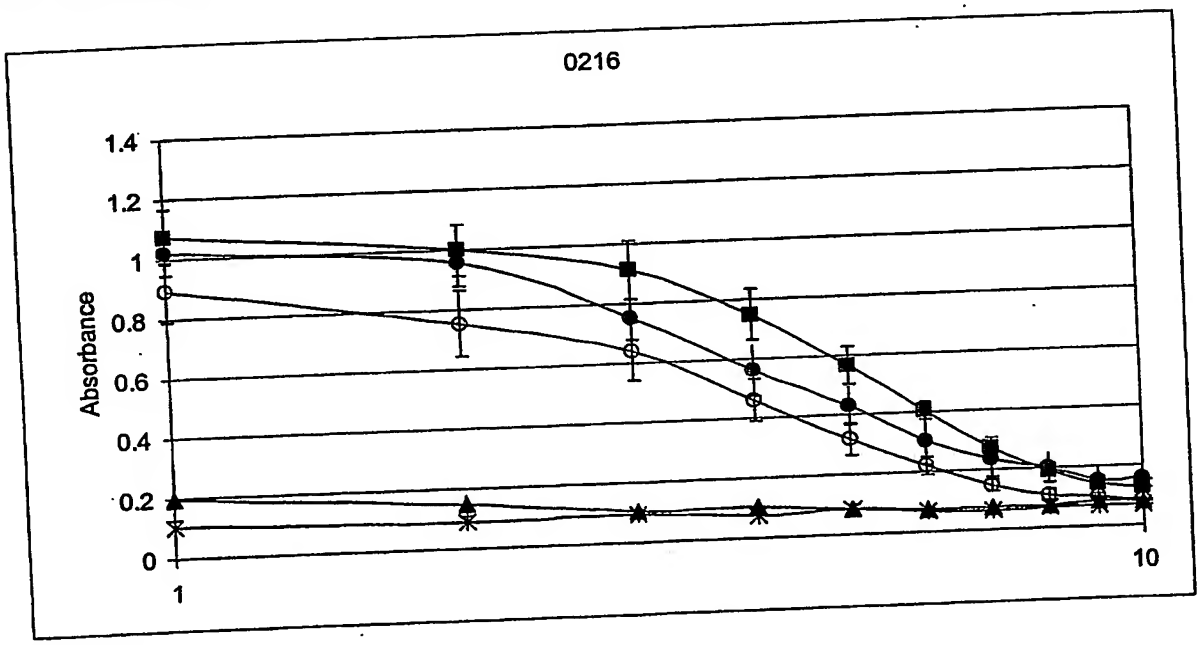


**Figure 7**

n=5 +/- SEM

PRIME
▲ DNA.HBs
○ DNA.HBs i.m. Engerix s.c.
● DNA.HBs i.m. Engerix s.c.
□ DNA.HBs i.m. Engerix s.c.
* Naive

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

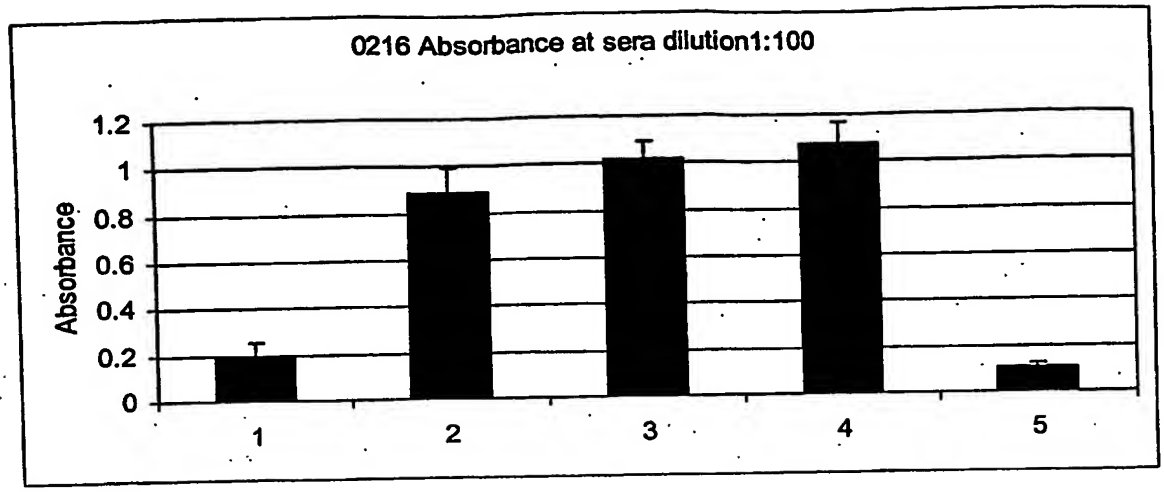


**Figure 8**

n=5 +/- SEM

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.
5. Naive

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

**MVA .LacZ and MVA.HBs boosts antibody responses equally**

DNA.HBs and Engerix-B priming combined with a boost of Engerix-B and either MVA.HBs(i.d or s.c) or MVA.LacZ s.c. increased antibody responses 10-fold compared to any immunisation regime in Experiment one.

The antibody responses to these three regimes were not significantly different from each other and it can therefore be concluded that MVA boosts antibody responses against HBsAg by acting as an adjuvant.

## 2.2 T-cell responses

### 2.2.1 Peptide Stimulated Splenocytes

DNA.HBs prime followed by MVA.HBs boost along with DNA.HBs and Engerix-B priming combined with a boost of Engerix-B s.c. and MVA.HBs i.d. give equally strong T-cell responses to peptide. However if MVA.HBs or MVA.LacZ is given s.c. with Engerix-B following DNA.HBs and Engerix-B priming this cellular response is abrogated. Therefore in order for strong cellular responses to be maintained the MVA needs to express the antigen present in the protein immunisation, in this case HBsAg. Priming with Engerix-B and DNA.HBs then boosting with MVA.HBs i.d. and Engerix-B s.c. significantly increases T-cell responses to peptide when compared with Exp 1 regime of DNA.HBs priming followed by boosting with MVA.HBs i.v. and Engerix-boost (p=0.004).

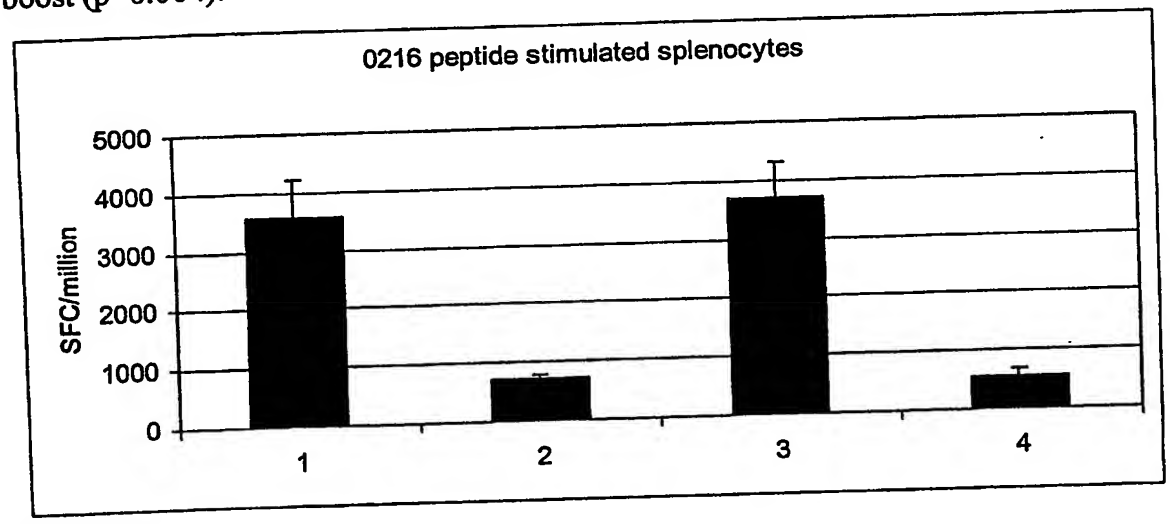


**Figure 9**

n=5 +/- SEM

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

## 2.2.2 HBsAg Stimulated Splenocytes

A similar pattern to that of peptide-stimulated splenocytes is seen upon HBsAg stimulation of splenocytes. The numbers are a lot lower than peptide stimulation. However, they are greater than those seen in experiment one.

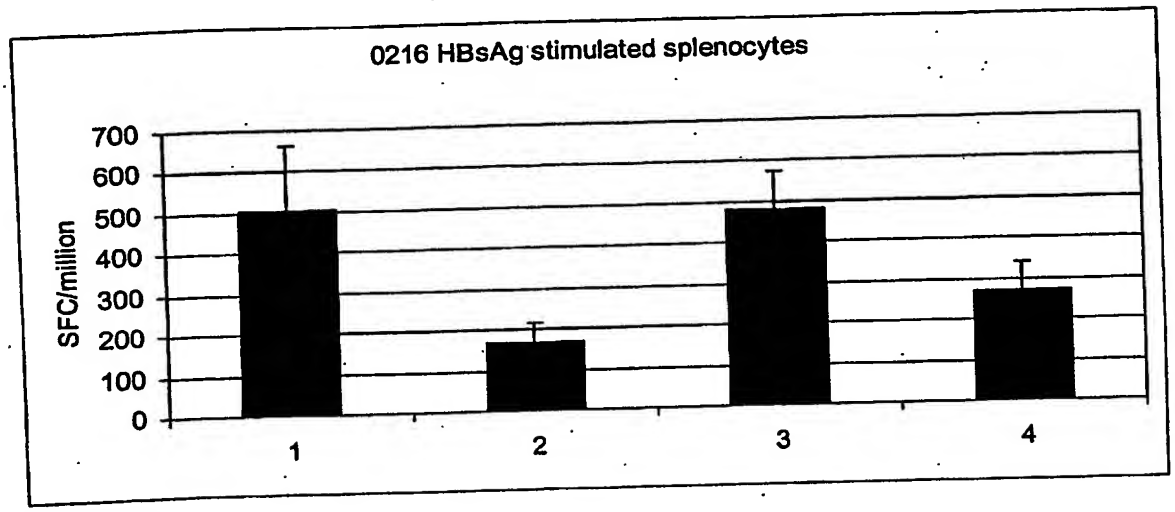


**Figure 10**

n=5 +/- SEM

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

### 2.2.3 Peptide Stimulated Lymph Nodes

As with Exp 1 when MVA.HBs is given at an alternate site to the Engerix boost the t-cell response is elevated in comparison to other immunisation regimes.

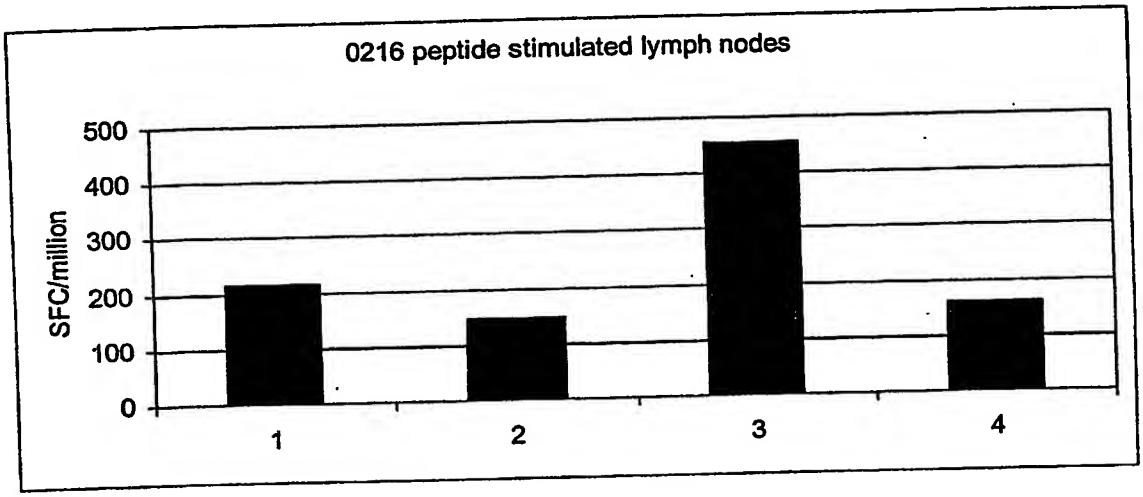


**Figure 11**

n= cells from 5 animals pooled

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

## 2.2.4 HBsAg Stimulated Lymph Nodes

Same pattern as peptide stimulated LN's, but half the numbers. Again a much higher response(11 fold) when MVA.HBs is given i.d. compared to s.c. with Engerix-B. Although, in Exp 1 DNA.HBs prime followed by MVA.HBS and Engerix-B boost s.c. is the only regime that gives good responses to HBsAg stimulated LN's.

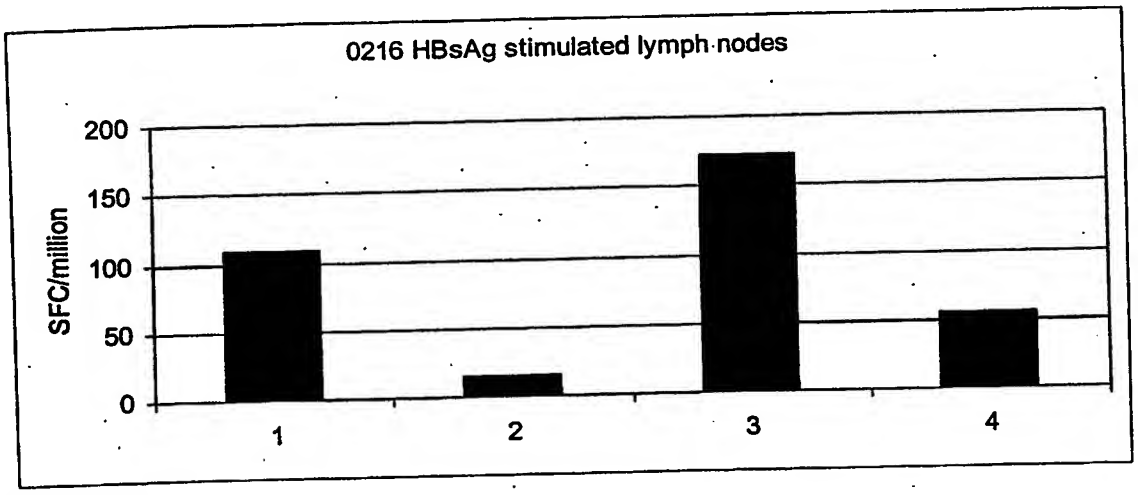


**Figure 12**

n= cells from 5 animals pooled

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

# Experiment 3

Aim:-

To establish whether a good cellular and Ab responses can be achieved in the absence of Alum using a homologous boost.

The same immunising agent and route was used for prime and boost.

## 3.1 Antibody responses

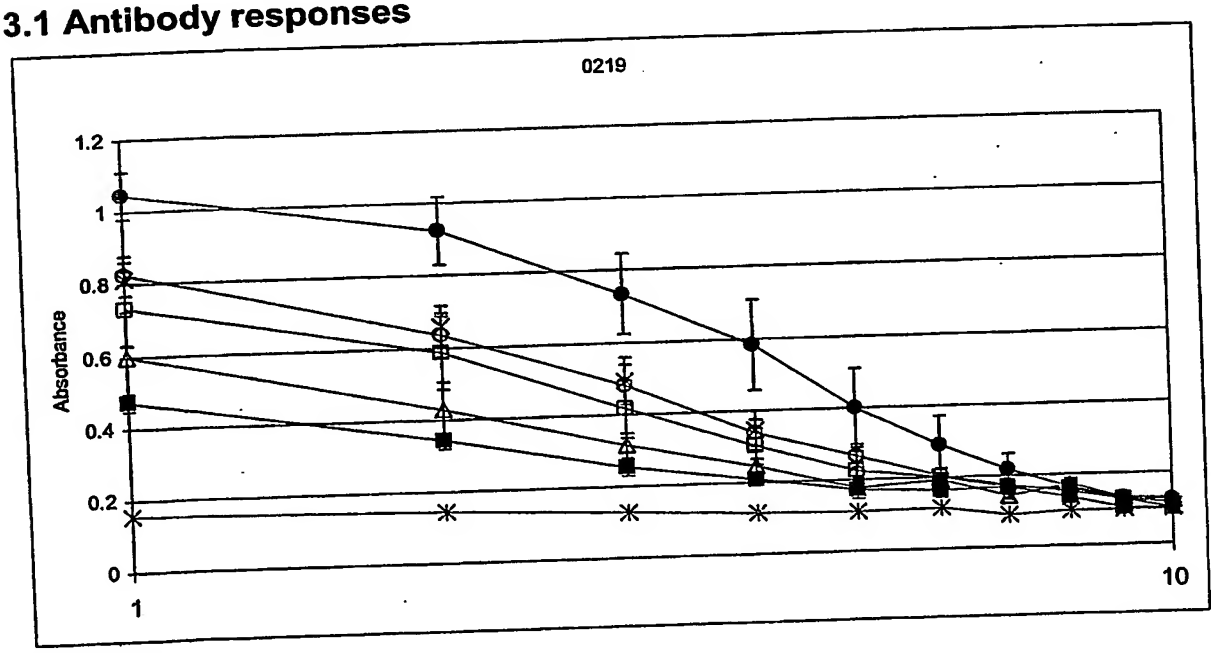


**Figure 13**

n=4 +/- SEM

PRIME
- ▲ HbsAg s.c.
- ○ HbsAg I.d.
- ● HbsAg + MVA.LacZ mixed i.d.
- □ Engerix-B s.c.
- ■ Eng-B s.c. MVA.LacZ i.d.
- × Eng-B + MVA.LacZ mix s.c.
- * Naïve

BOOST
- HbsAg s.c.
- HbsAg I.d.
- HbsAg + MVA.LacZ mixed i.d.
- Engerix-B s.c.
- Eng-B s.c. MVA.LacZ i.d.
- Eng-B + MVA.LacZ mix s.c.

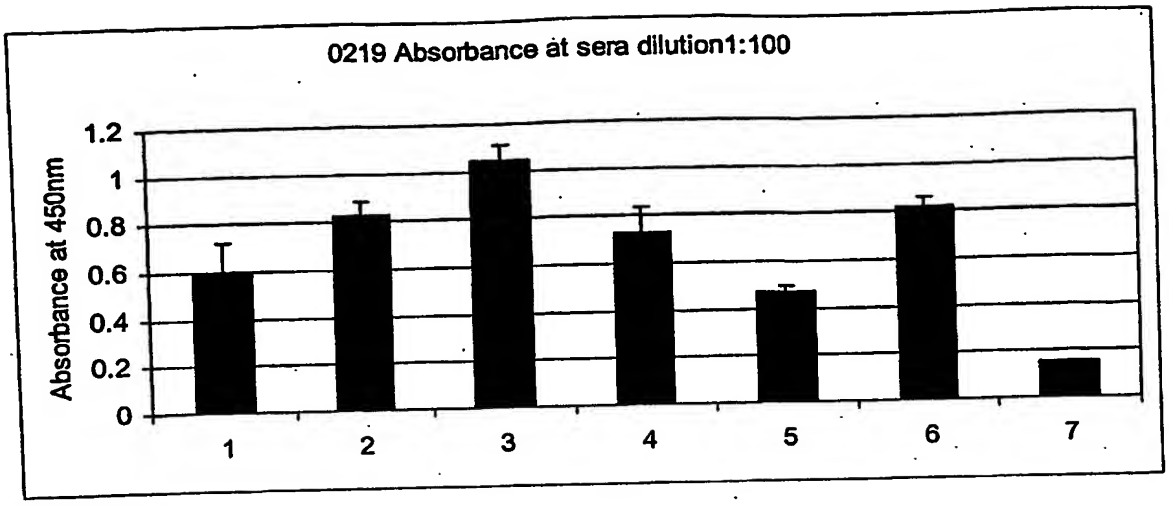


**Figure 14**

n=4 +/- SEM

PRIME
1.HbsAg s.c.
2.HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.
7.Naïve

BOOST
HbsAg s.c.
HbsAg i.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

Highest level of antibodies were achieved with HBsAg mixed with MVA.LacZ and administered i.d. Levels were not significantly different to Exp 2 immunisations where DNA.HBs priming was followed by boosting with Engerix-B and MVA.HBs (i.d. or s.c.) or MVA.LacZ s.c. ($p= 0.905$, 0.19 and 0.905 respectively).

Two immunisations with Engerix-B produced lower antibody responses than HBsAg mixed with MVA.LacZ administered i.d ($p= 0.057$).

Homologous immunisation with HBsAg i.d. alone was not significantly different from Engerix-B alone or HBsAg and MVA.LacZ given i.d., ($p= 0.686$ and 0.114 respectively).

Engerix-B given with MVA.LacZ s.c (prime and boost) was significantly lower in two groups from Exp 1 that received DNA.HBs and Engerix-B priming combined with a boost of Engerix-B and either MVA.HBs i.d.. or MVA.LacZ s.c ($p= 0.032$ both). But not if the MVA.HBs was given s.c..($p=0.556$)

This indicates that DNA.HBs priming increases the antibody response to HBsAg .

High levels of antibody can be induced by priming with DNA.HBs and Engerix-B then boosting with MVA.LacZ or MVA.HBs and Engerix-B. These levels are comparable to those induced by two homologous immunisations of HBsAg and MVA.LacZ i.d.

## 3.2 T-cell Responses

### 3.2.1 Peptide Stimulated Splenocytes

T-cell responses to all vaccine regimes were very low(all well below 500 SFC/million) when compared to the best regimes of other Experiments.

Surprisingly, homologous prime/boost of Engerix-B mixed with MVA.LacZ and administered s.c yielded similar numbers to the **Spec2 ???** regime of DNA.HBs priming followed by boosting with Engerix-B and MVA.LacZ s.c. (average of 336 +/-113 SEM and 511 +/-173 SEM SFC/million respectively). This suggests that DNA.HBs combined with Engerix-B priming is not enough to increase cellular responses to HBsAg when the MVA combined with Engerix-B is non-specific, in this case MVA.LacZ given either s.c. or i.d. The MVA administered at the time of boosting needs to contain HBs in order to attain high levels of specific T-cells.

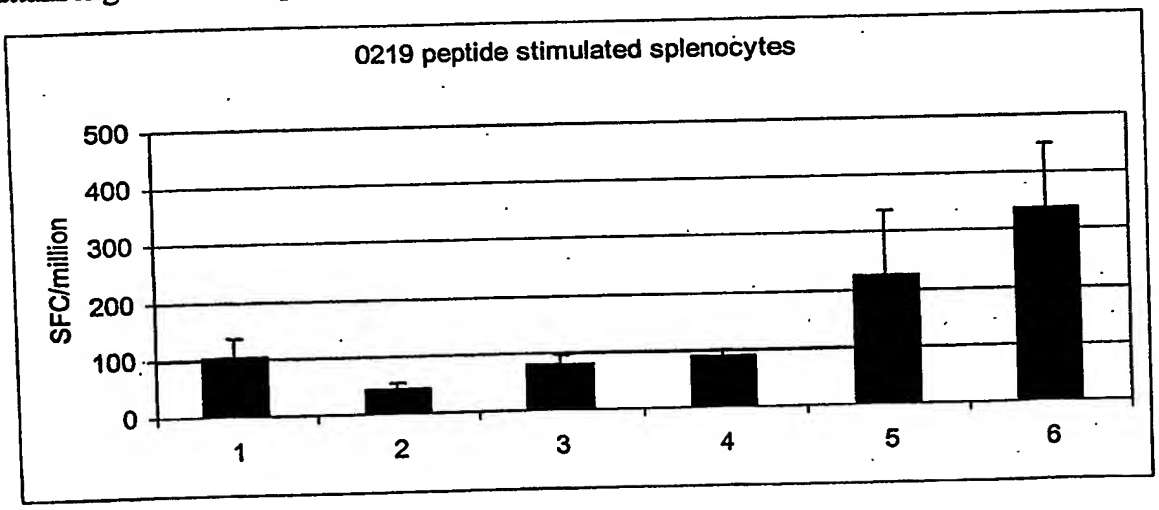


**Figure 15**
n=4 +/- SEM

PRIME
1.HbsAg s.c.
2.HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.

BOOST
HbsAg s.c.
HbsAg i.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

### 3.2.2 HBsAg Stimulated Splenoctyes

All below 150 SFC/million

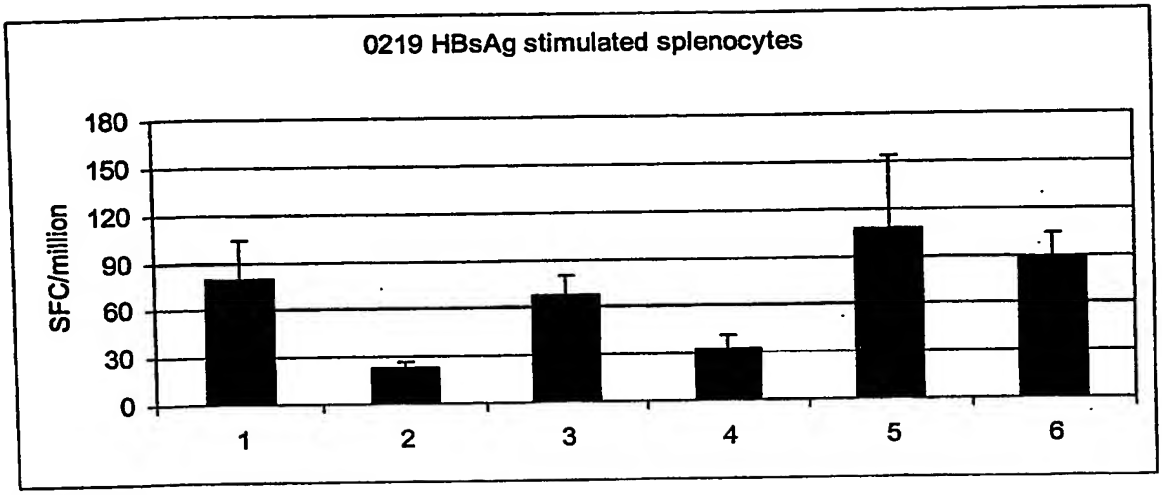


**Figure 16**

n=4 +/- SEM

PRIME
1.HbsAg s.c.
2.HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.

BOOST
HbsAg s.c.
HbsAg i.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

### 3.2.3 Peptide Stimulated Lymph Nodes

Kept Axial and Cervical Lymph nodes separately. s.c. immunisations more likely to predominantly drain to the Axial LN's, i.d immunisations more likely to predominantly drain to cervical L.N's. HBsAg alone s.c gives higher responses in Axial L.N's. When given i.d. Cervical LN's have higher responses, equal to level in Axial LN's following s.c.immunisation.

Favourable regimes were either HBsAg mixed with MVA.LacZ and given i.d. or Engerix-B mixed with LacZ and given s.c.(363 and 343 respectively) in cervical LN's. Engerix-B given s.c. and MVA.LacZ given i.d. produced almost equal numbers of spots in cervical and axial LN's(147 and 132 respectively) and was similar in number to Engerix-B alone(83 cervical and 167 axial). This suggested that MVA.lacZ did not adjuvant Engerix-B in LN's when given i.d. but did in cervical LN's if mixed with Engerix-B and given s.c, therefore scenario the antigen of interest may need to be immunised at the same site as the MVA.LacZ to be adjuvanted by it.

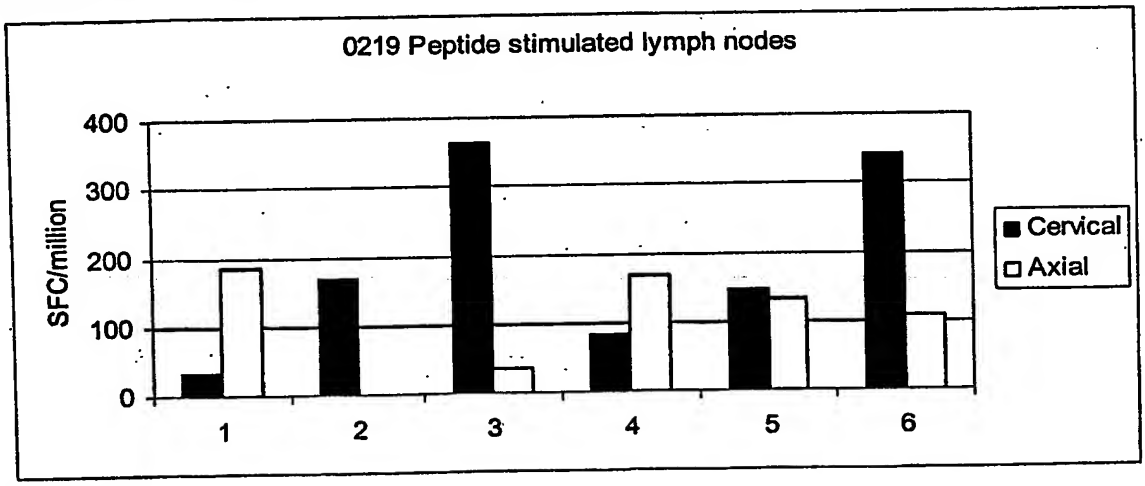


**Figure 17**
n= cells from 4 animals pooled

PRIME
1.HbsAg s.c.
2.HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.

BOOST
HbsAg s.c.
HbsAg i.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

### 3.2.4 HBsAg Stimulated Lymph Nodes

Similar pattern to peptide stimulation of LN's except even larger response in cervical LN's following i.d. immunisation of HBsAg mixed with MVA.LacZ(828 SFC/million).

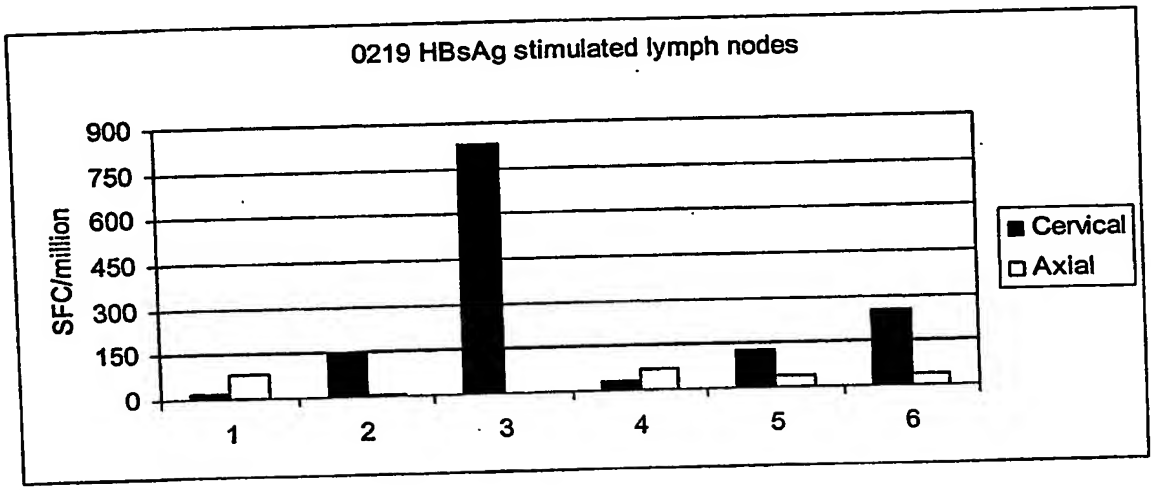


**Figure 18**

n= cells from 4 animals pooled

PRIME
1.HbsAg s.c.
2.HbsAg I.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.

BOOST
HbsAg s.c.
HbsAg I.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

## Conclusions From Experiments 1-3

DNA priming was shown to be important for inducing cellular and to a lesser extent antibody responses in specs 1-3.
Exp 1 showed DNA.HBs priming followed by boosting with Engerix-B and MVA gave good cellular responses but low Ab levels.
Exp 2 showed that by including an Engerix-b prime with DNA then boosting with Engerix-B and MVA gave high T-cell and antibody levels

**Exp 3 showed homologous prime/boost with MVA and HBsAg gave strong Ab responses but very low T-cell responses.**

**Antibody responses**

a) MVA.LacZ and MVA.HBs can adjuvant the protein HBsAg to induce high levels of specific antibody against HBsAg.
b) DNA.HBs priming increases the antibody response to HBsAg when Engerix-B s.c. is concurrently used to prime followed by boosting with Engerix-B and MVA.lacZ s.c. (Experiment 3 group 4 vs Spec 3 group 6)
c) MVA.LacZ adjuvants Engerix-B to produce high antibody levels when both are mixed and immunised s.c. (Experiment 3, group 5 vs group 6)

**T-Cell responses**

a) DNA.HBs priming is required to induce high levels of specific T-cells. (Exp 3 vs Exp 1 & 2)
b) Following DNA priming highest T-cell responses are achieved by administering MVA.HBs i.v. or i.d, not s.c. (Spec 2)
c) Engerix-B used at prime with DNA HBs increases T-cell responses when boosted with MVA.HBs i.v. and Engerix-B s.c. (Spec 2 group 3 vs Spec 1 group 4)

**A favourable strategy for inducing Antibody and T-cells to date:**

Prime: DNA.HBs i.m. and Engerix-B s.c.
Boost: MVA.HBs i.d. and Engerix-B s.c.

T-cell responses: Not significantly different (P =0.61) from our 'gold standard' T-cell inducing regiment DNA.HBs i.m. prime and MVA.HBs i.v. or i.d. boost.

Antibody responses: Higher antibody levels than the 'gold standard' of homologous prime/boost with Engerix-B, of marginal statistical significance (p=0.06)

MVA may direct the immune system towards epitopes not normally targeted in current vaccine regimes thus overcoming the problem of non-responders.

# Experiment 4

Aim: to retain HBsAg and MVA boost giving a good Ab responses, whilst improving the prime to restore cellular responses. Furthermore, an aim of this expereiment was to establisgh whether the strong cellular response restored if MVA.HBs is used to adjuvant HBsAg at prime and boost, and whether FP.LacZ has an adjuvant activity for antibody induction.

## 4.1 Antibody Responses

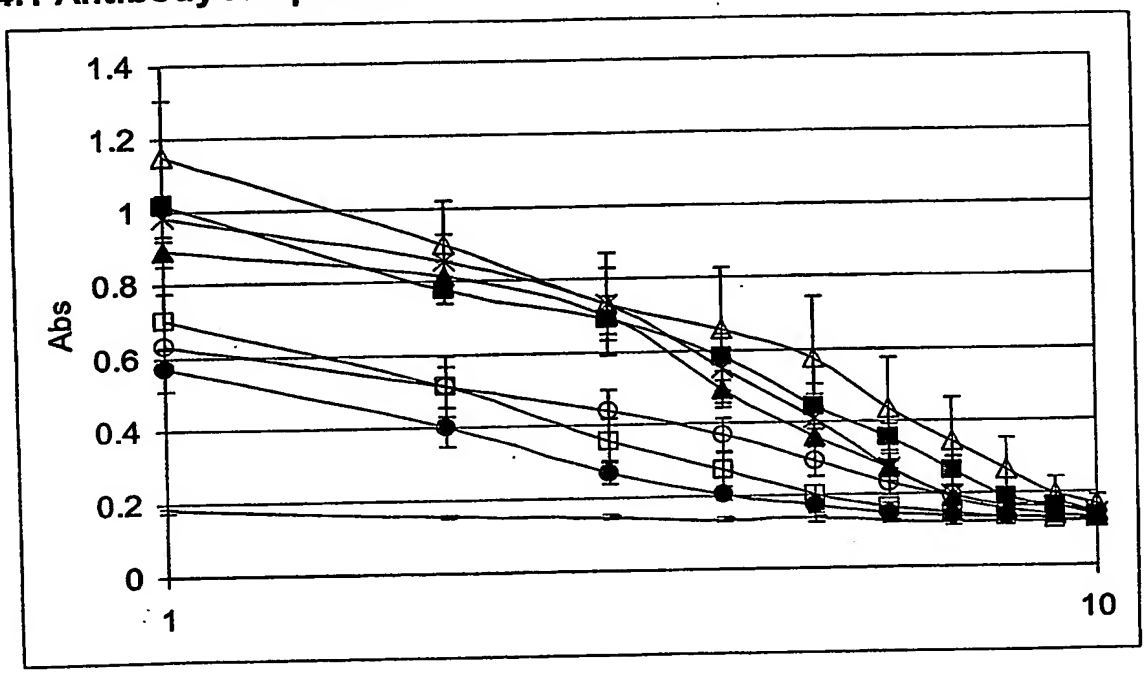


**Figure 19**

n=4 +/- SEM

PRIME
Δ HBsAg + MVA.HBs mix i.d.
▲ DNA.HBs + HBsAg mix i.d.
□ Engerix-B 5ug s.c.
■ DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
○ DNA.HBs i.m. & HBsAg i.d..
● DNA.HBs i.m. & Engerix-B s.c.
* HBsAg + MVA.LacZ mix i.d.
— Naive

BOOST
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
Engerix-B 5ug s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
Engerix-B s.c. + MVA.HBs i.d.
HBsAg + FP9.LacZ mix i.d.

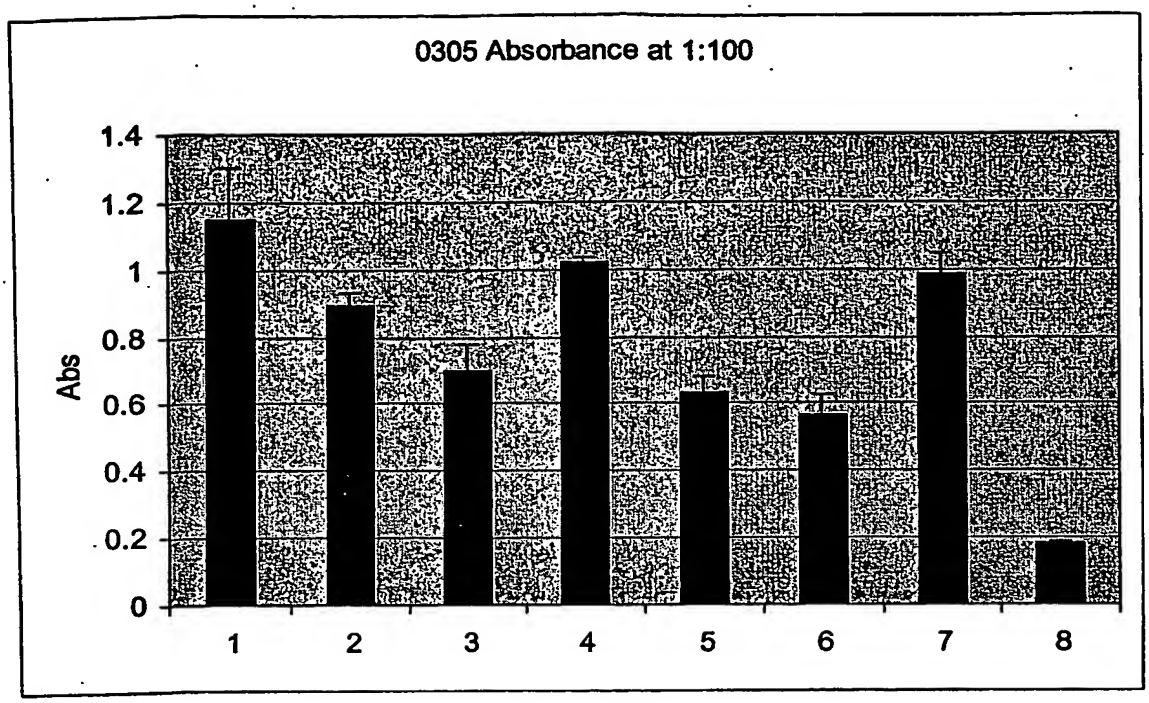


**Figure 20**

n=4 +/- SEM

| PRIME | BOOST |
|---|---|
| 1 HBsAg + MVA.HBs mix i.d. | HBsAg + MVA.HBs mix i.d |
| 2 DNA.HBs + HBsAg mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3 Engerix-B 5ug s.c. | Engerix-B 5ug s.c. |
| 4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 5 DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 6 DNA.HBs i.m. & Engerix-B s.c. | Engerix-B s.c. + MVA.HBs i.d. |
| 7 HBsAg + MVA.LacZ mix i.d. | HBsAg + FP9.LacZ mix i.d. |
| 8. Naive | |

## 4.2 T-cell Responses

### 4.2.1 Peptide Stimulated Splenocytes

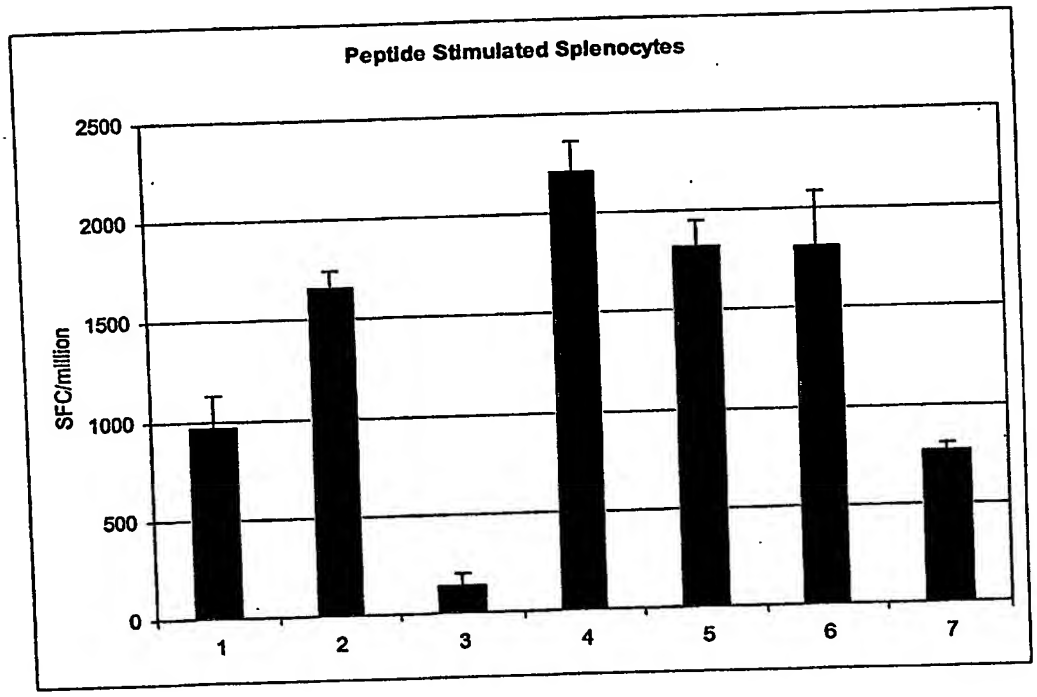


**Figure 21**

n=4 +/- SEM

PRIME
1 HBsAg + MVA.HBs mix i.d.
2 DNA.HBs + HBsAg mix i.d.
3 Engerix-B 5ug s.c.
4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
5 DNA.HBs i.m. & HBsAg i.d.
6 DNA.HBs i.m. & Engerix-B s.c.
7 HBsAg + MVA.LacZ mix i.d.

BOOST
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
Engerix-B 5ug s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
Engerix-B s.c. + MVA.HBs i.d.
HBsAg + FP9.LacZ mix i.d.

## 4.2.2 HBsAg stimulated splenocytes

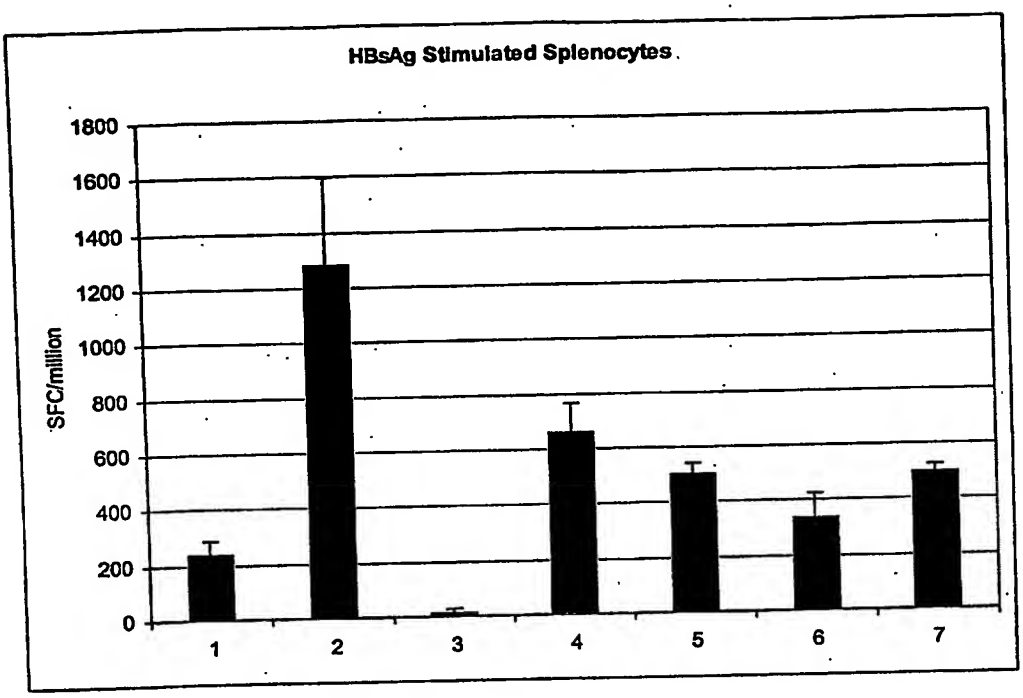


**Figure 22**

n=4 +/- SEM

PRIME
1 HBsAg + MVA.HBs mix i.d.
2 HBsAg + pSG2.HBs mix i.d.
3 Engerix-B 5ug s.c.
4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
5 DNA.HBs i.m. & HBsAg i.d.
6 DNA.HBs i.m. & Engerix-B s.c.
7 HBsAg + MVA.LacZ mix i.d.

BOOST
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
Engerix-B 5ug s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
Engerix-B s.c. + MVA.HBs i.d.
HBsAg + FP9.LacZ mix i.d.

## 4.2.3 Peptide Stimulated lymph nodes

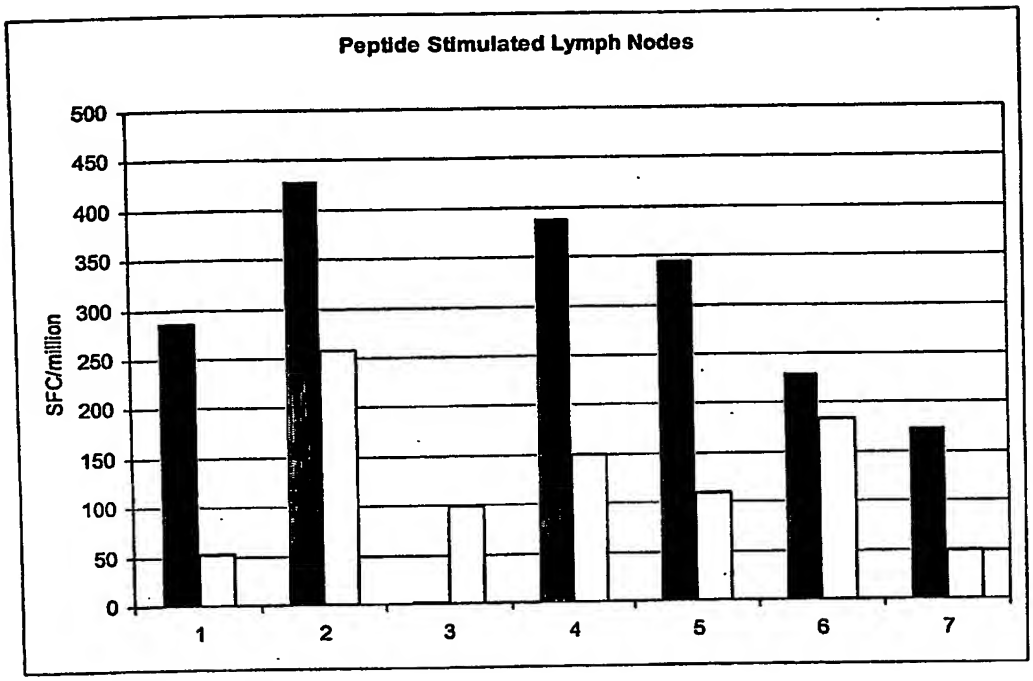


**Figure 23**

n= cells from 4 animals pooled

| PRIME | BOOST |
|---|---|
| 1 HBsAg + MVA.HBs mix i.d. | HBsAg + MVA.HBs mix i.d |
| 2 DNA.HBs + HBsAg mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3 Engerix-B 5ug s.c. | Engerix-B 5ug s.c. |
| 4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 5 DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 6 DNA.HBs i.m. & Engerix-B s.c. | Engerix-B s.c. + MVA.HBs i.d. |
| 7 HBsAg + MVA.LacZ mix i.d. | HBsAg + FP9.LacZ mix i.d. |

## 4.2.4 HBsAg Stimulated lymph nodes

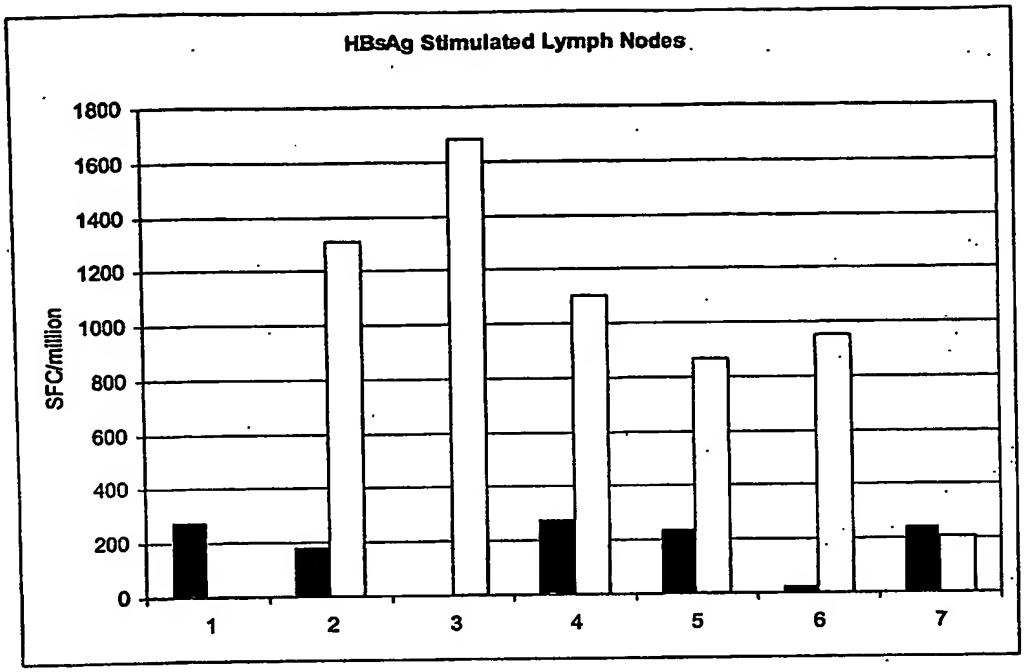


**Figure 24**

n= cells from 4 animals pooled

| PRIME | BOOST |
|---|---|
| 1 HBsAg + MVA.HBs mix i.d. | HBsAg + MVA.HBs mix i.d |
| 2 DNA.HBs + HBsAg mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3 Engerix-B 5ug s.c. | Engerix-B 5ug s.c. |
| 4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 5 DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 6 DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs i.d. + Engerix-B s.c. |
| 7 HBsAg + MVA.LacZ mix i.d. | HBsAg + FP9.LacZ mix i.d. |

**Experiment 4 demonstrates that:**

1. FP9 has adjuvant activity for antibody induction to the co-administered antigen – compared groups 4 and 5.
2. A heterologous prime-boost regime is required for optimal T cell induction; compare T cell responses in spleen in groups 1 and 2.

# Summary of results from experiments 1-4

For ease of reference, the results of experiments 1-4 are compread, using the followiung key:

| PRIME | BOOST |
|---|---|
| 1. DNA.HBs i.m. | MVA.HBs i.v. |
| 2. Nil | Engerix-B s.c. |
| 3. DNA.HBs i.m. | MVA.HBs + Engerix-B s.c. |
| 4. DNA.HBs i.m. | MVA.HBs i.v. Engerix-B s.c. |
| 5. DNA.HBs i.m. | Engerix-B s.c. |
| 6. DNA.HBs i.m. | MVA.HBs + Alum s.c. |
| 7. DNA.HBs | MVA.HBs i.d. |
| 8. DNA.HBs i.m. Engerix s.c. | MVA.HBs s.c. Engerix s.c. |
| 9. DNA.HBs i.m. Engerix s.c. | MVA.HBs i.d. Engerix s.c. |
| 10. DNA.HBs i.m. Engerix s.c. | MVA.lacZ s.c. Engerix s.c. |
| 11. HbsAg s.c. | HbsAg s.c. |
| 12. HbsAg I.d. | HbsAg I.d. |
| 13. HbsAg + MVA.LacZ mixed i.d. | HbsAg + MVA.LacZ mixed i.d. |
| 14. Engerix-B s.c. | Engerix-B s.c. |
| 15. Eng-B s.c. MVA.LacZ i.d. | Eng-B s.c. MVA.LacZ i.d. |
| 16. Eng-B + MVA.LacZ mix s.c. | Eng-B + MVA.LacZ mix s.c. |
| 17. HBsAg + MVA.HBs mix i.d. | HBsAg + MVA.HBs mix i.d |
| 18. HBsAg + pSG2.HBs mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 19. Engerix-B 5ug s.c. | Engerix-B 5ug s.c. |
| 20. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 21. DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 22. DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs i.d. + Engerix-B s.c. |
| 23. HBsAg + MVA.LacZ mix i.d. | HBsAg + FP9.LacZ mix i.d. |

**Antibody responses**

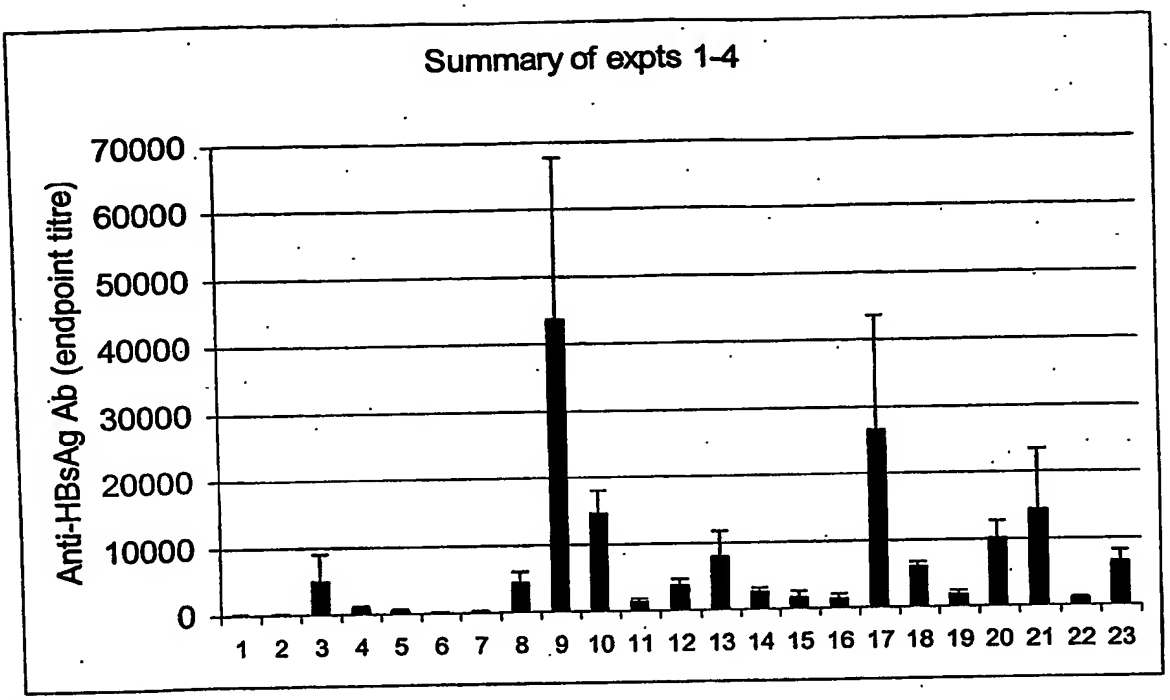


**Figure 25**

n=3-6 +/- SEM

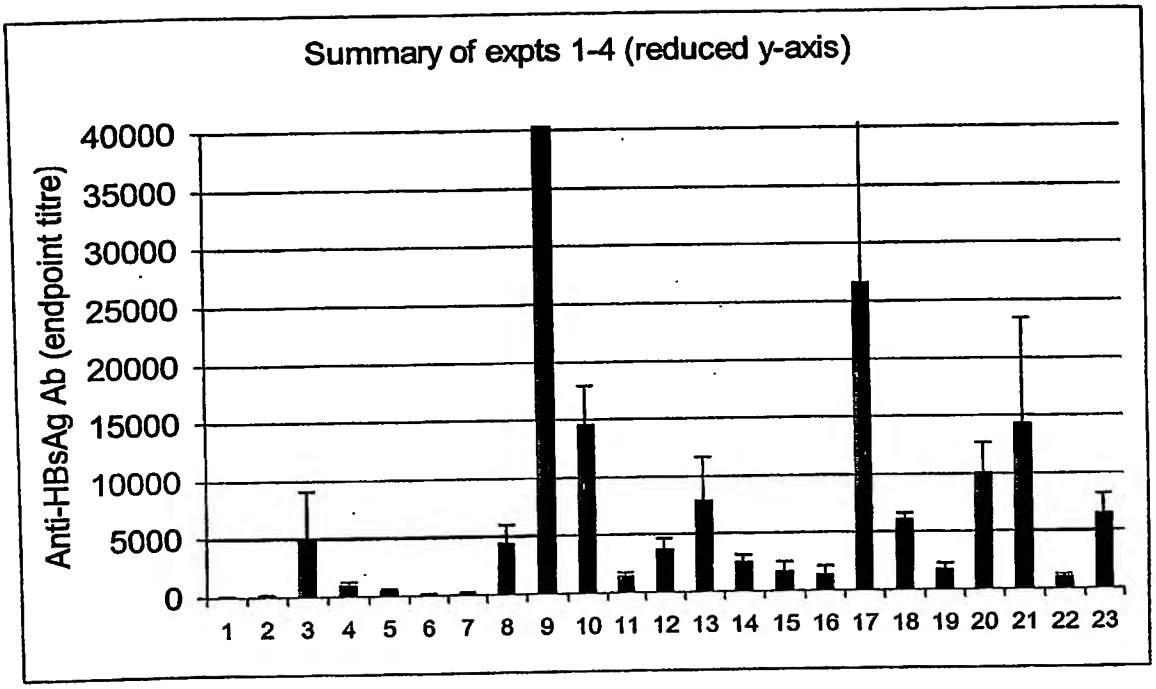


**Figure 26**

n=3-6 +/- SEM

**Cellular responses**

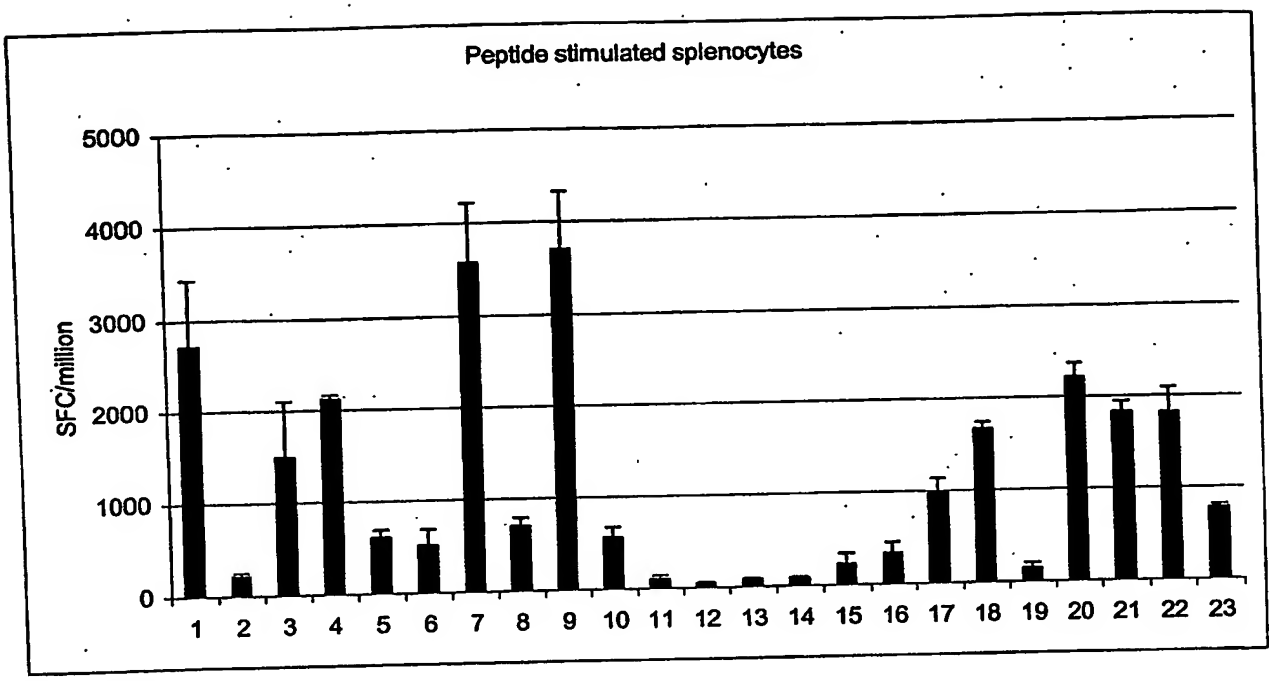


**Figure 27**

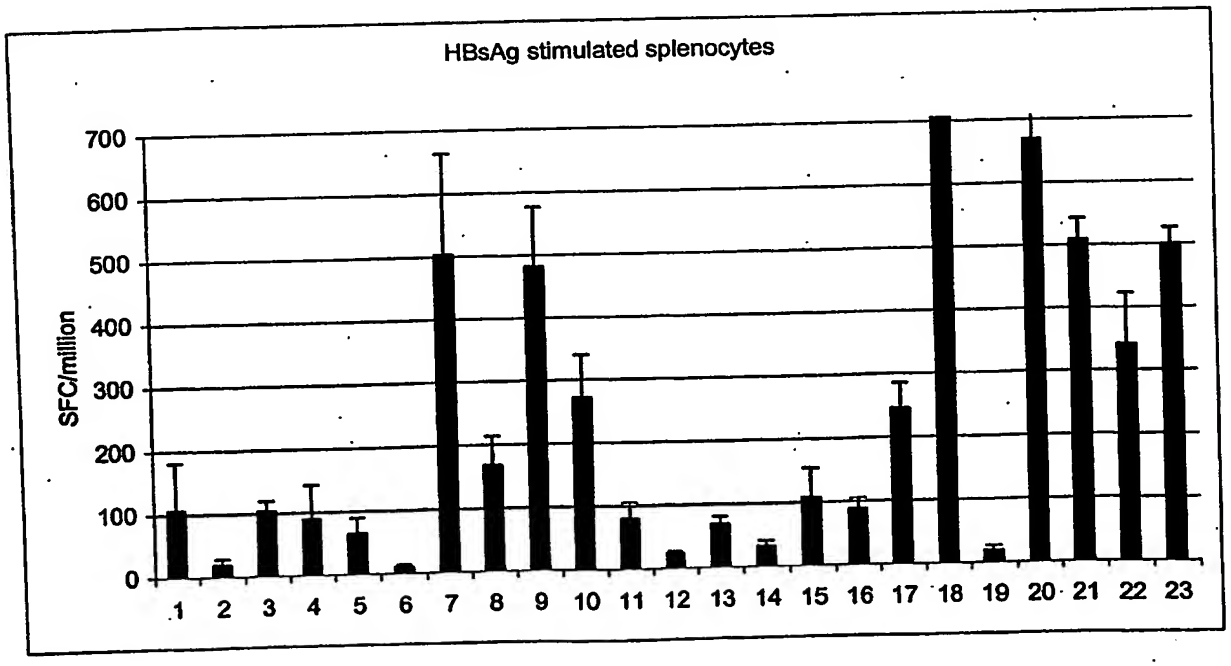


**Figure 28**

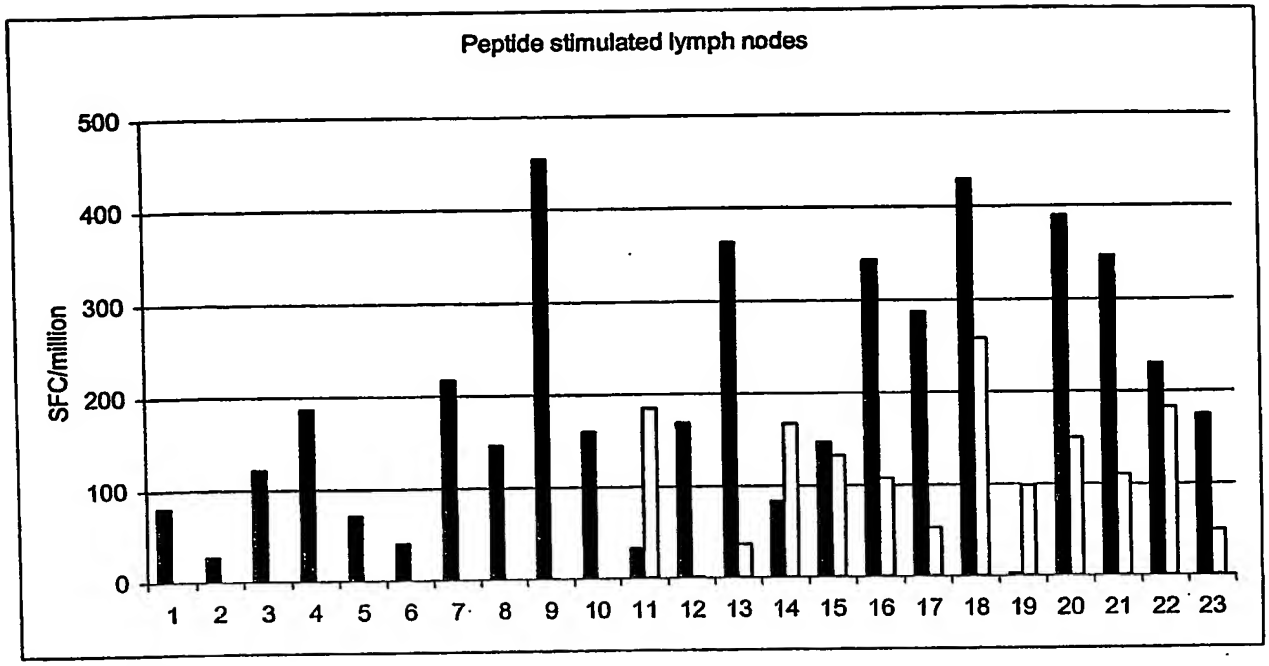


**Figure 29**

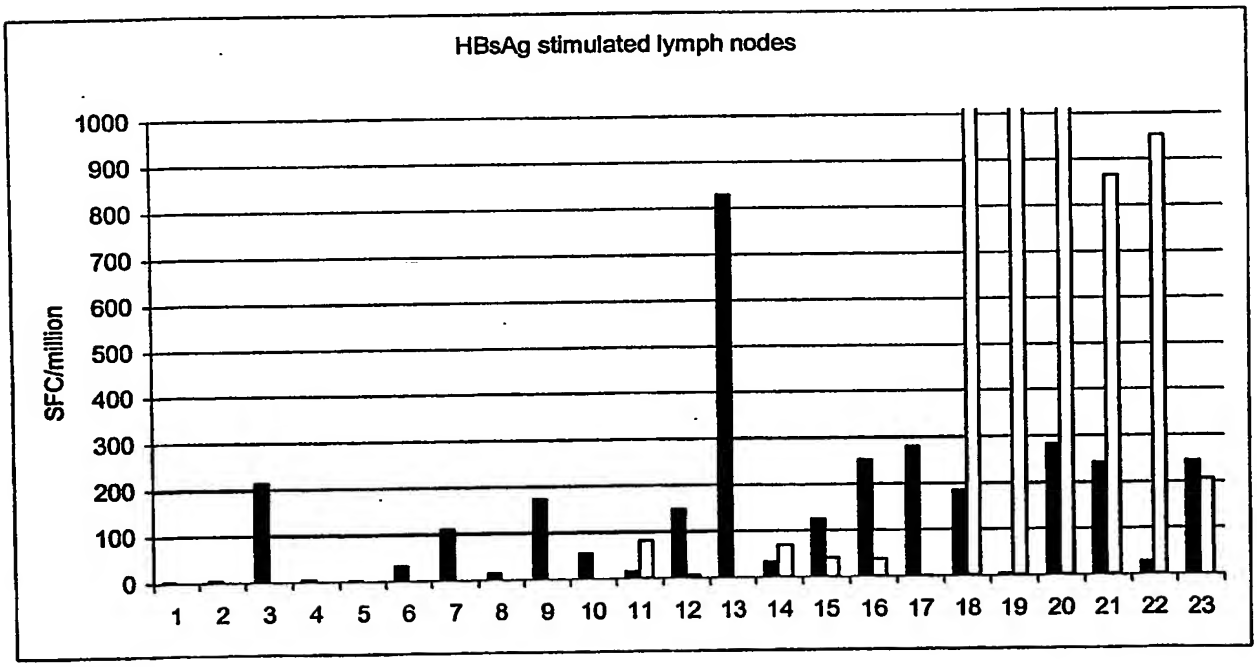


**Figure 30**

# Experiment 5

Favourable antibody and cellular inducing regimes from experiments 1-4 were compared.

## 5.1 Antibody Responses

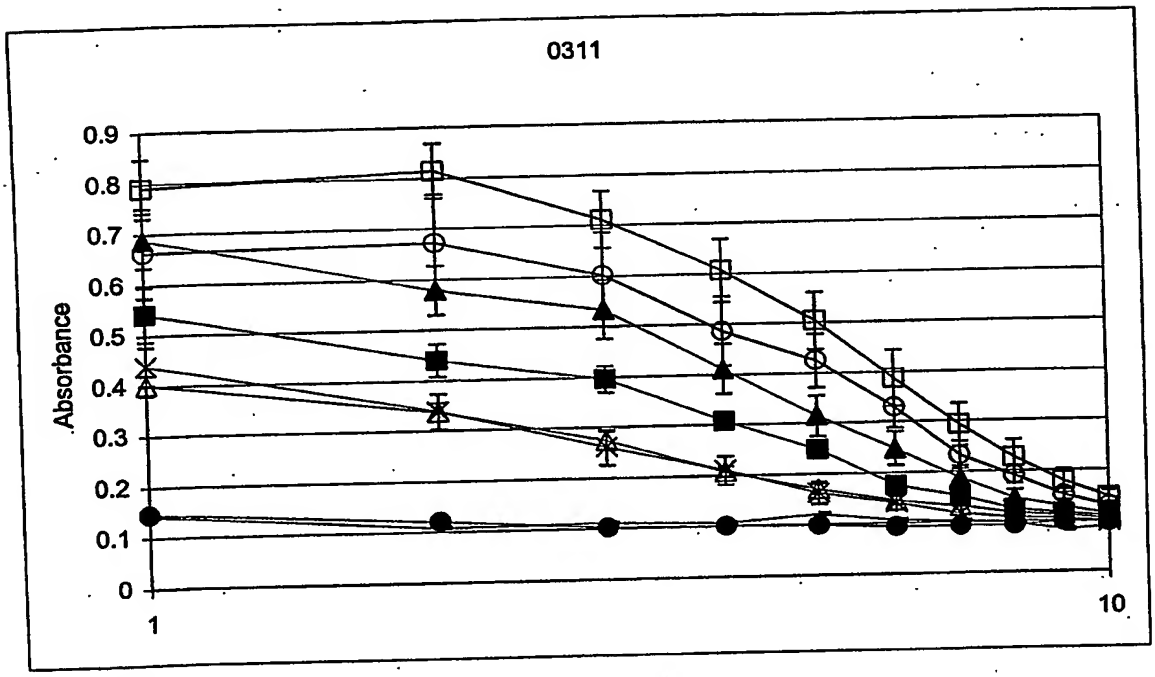


**Figure 31**

n=4 +/- SEM

PRIME
- Δ DNA.HBs i.m. Engerix s.c.
- ▲ HBsAg + pSG2.HBs mix i.d.
- □ DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
- ■ DNA.HBs i.m. & HBsAg i.d.
- ○ HBsAg + MVA.HBs mix i.d
- ● DNA.HBs i.m.
- * Engerix-B s.c.
- — Naive

BOOST
- MVA.HBs i.d. Engerix s.c.
- HBsAg + MVA.HBs mix i.d.
- HBsAg + MVA.HBs mix i.d
- HBsAg + MVA.HBs mix i.d.
- HBsAg + MVA.HBs mix i.d
- MVA.HBs i.d
- Engerix-B s.c.

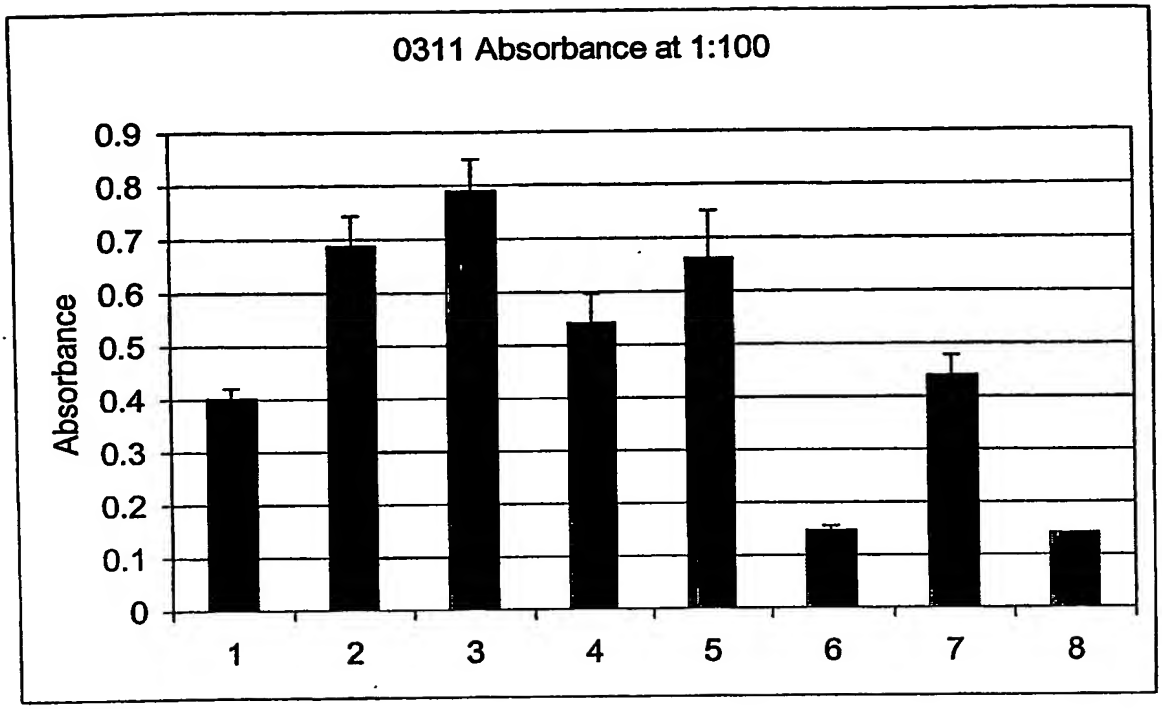


**Figure 32**

n=3-4 +/- SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.
8. Naive

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

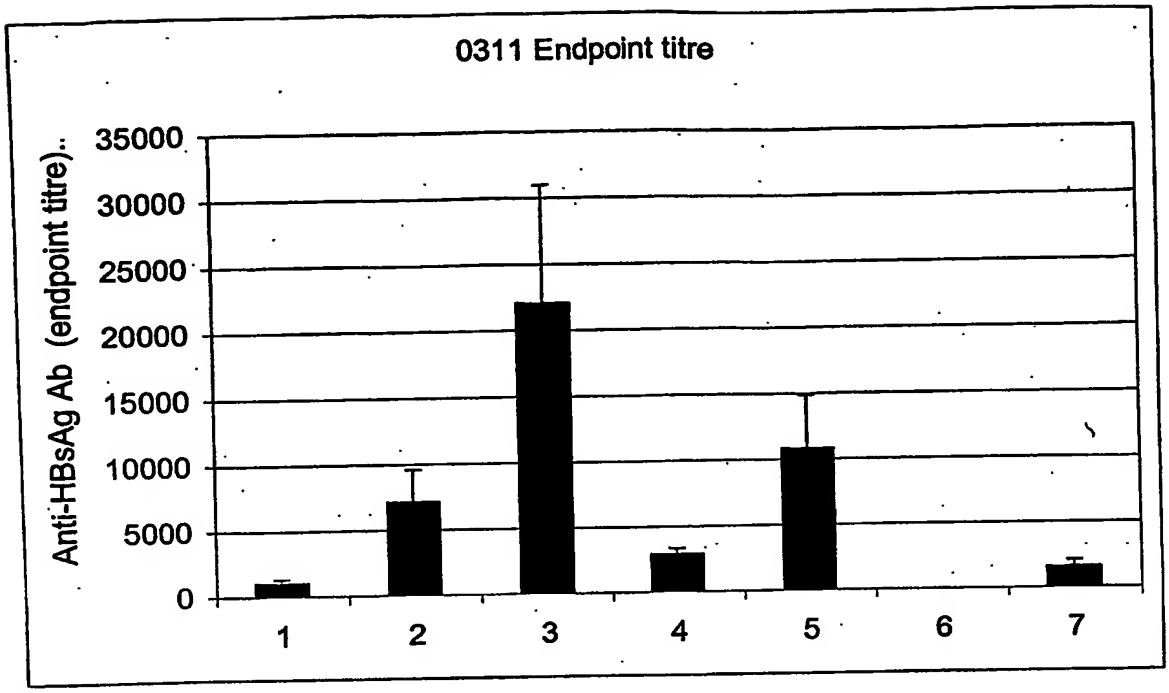


**Figure 33**

n=3-4 +/- SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.
8. Naive

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

## 5.2 T-cell Responses

### 5.2.1 Peptide Stimulated Splenocytes

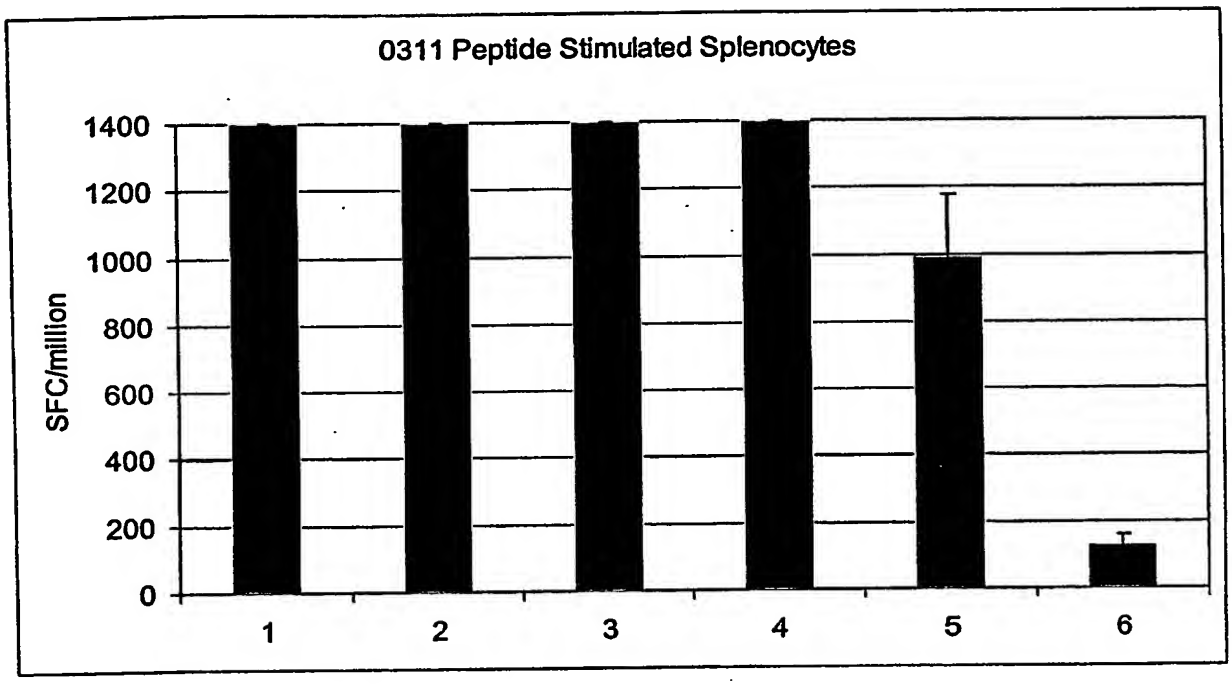


**Figure 34**

n=3-4 +/- SEM

| PRIME | BOOST |
|---|---|
| 1. DNA.HBs i.m. Engerix s.c. | MVA.HBs i.d. Engerix s.c. |
| 2. HBsAg + pSG2.HBs mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d |
| 4. DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 5. HBsAg + MVA.HBs mix i.d | HBsAg + MVA.HBs mix i.d |
| 6. Engerix-B s.c. | Engerix-B s.c. |

## 5.2.2 HBsAg stimulated splenocytes

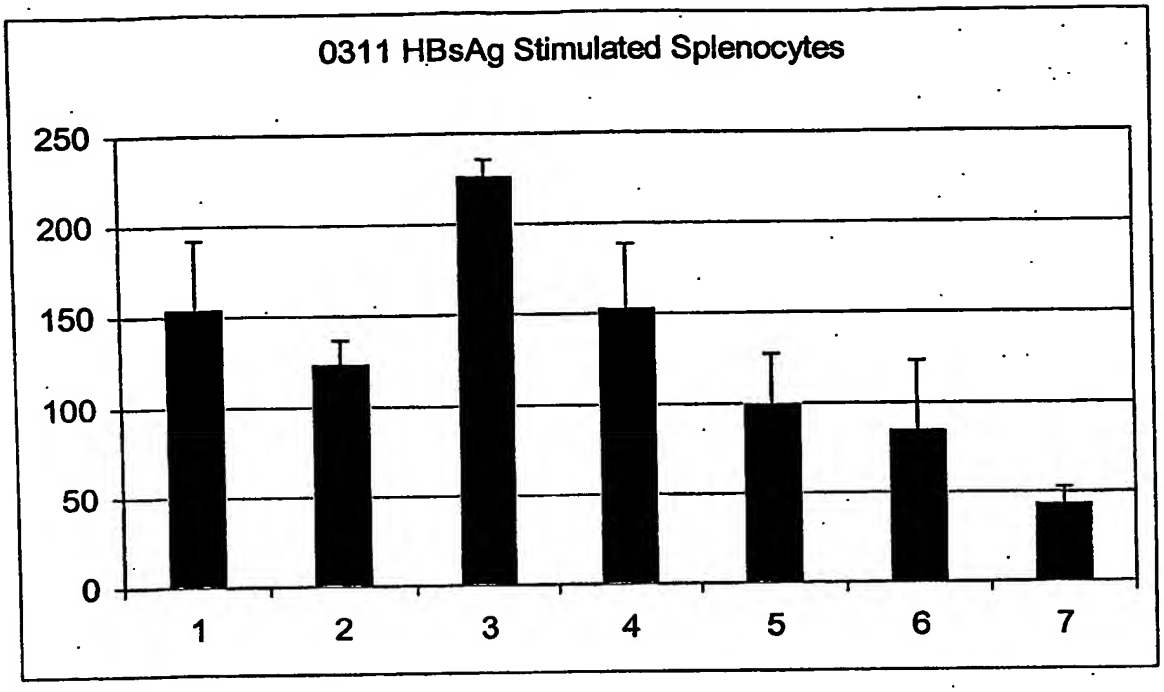


**Figure 35**
n=3-4 +/- SEM

| PRIME | BOOST |
|---|---|
| 1. DNA.HBs i.m. Engerix s.c. | MVA.HBs i.d. Engerix s.c. |
| 2. HBsAg + pSG2.HBs mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d |
| 4. DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 5. HBsAg + MVA.HBs mix i.d | HBsAg + MVA.HBs mix i.d |
| 6. DNA.HBs i.m. | MVA.HBs i.d |
| 7. Engerix-B s.c. | Engerix-B s.c. |

## 5.2.3 Peptide Stimulated Lymph Nodes

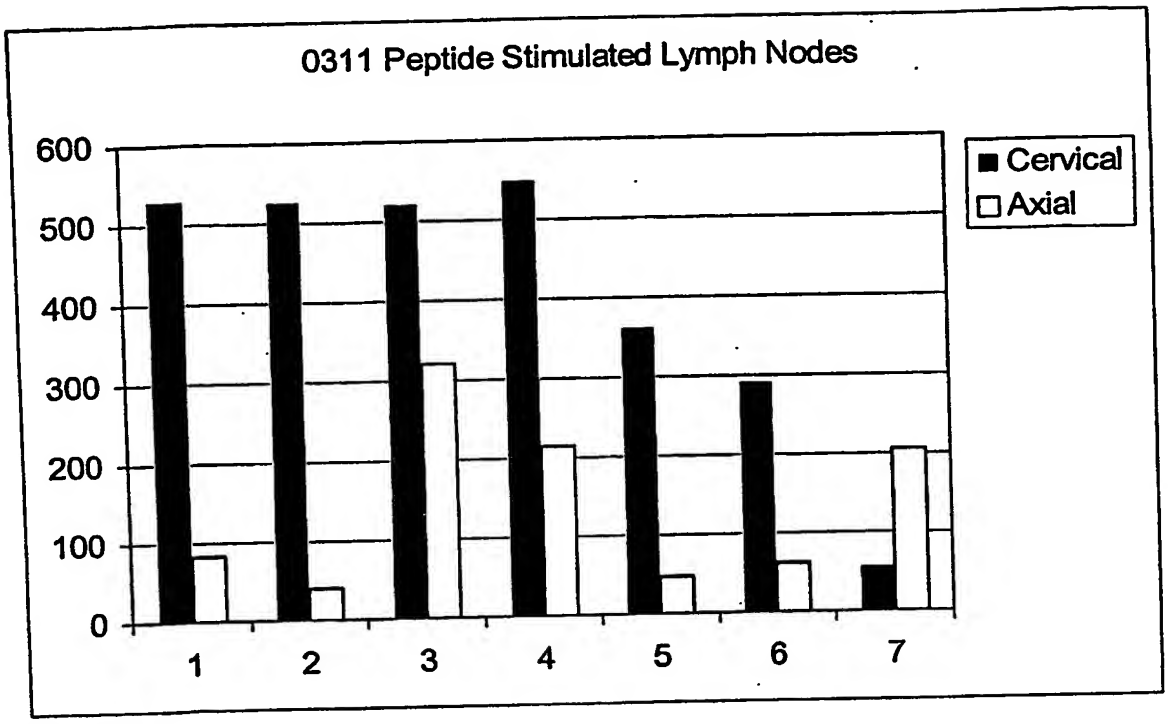


**Figure 36**
n=pooled lymph nodes from 3-4 animals

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

## 5.2.4 HBsAg Stimulated Lymph Nodes

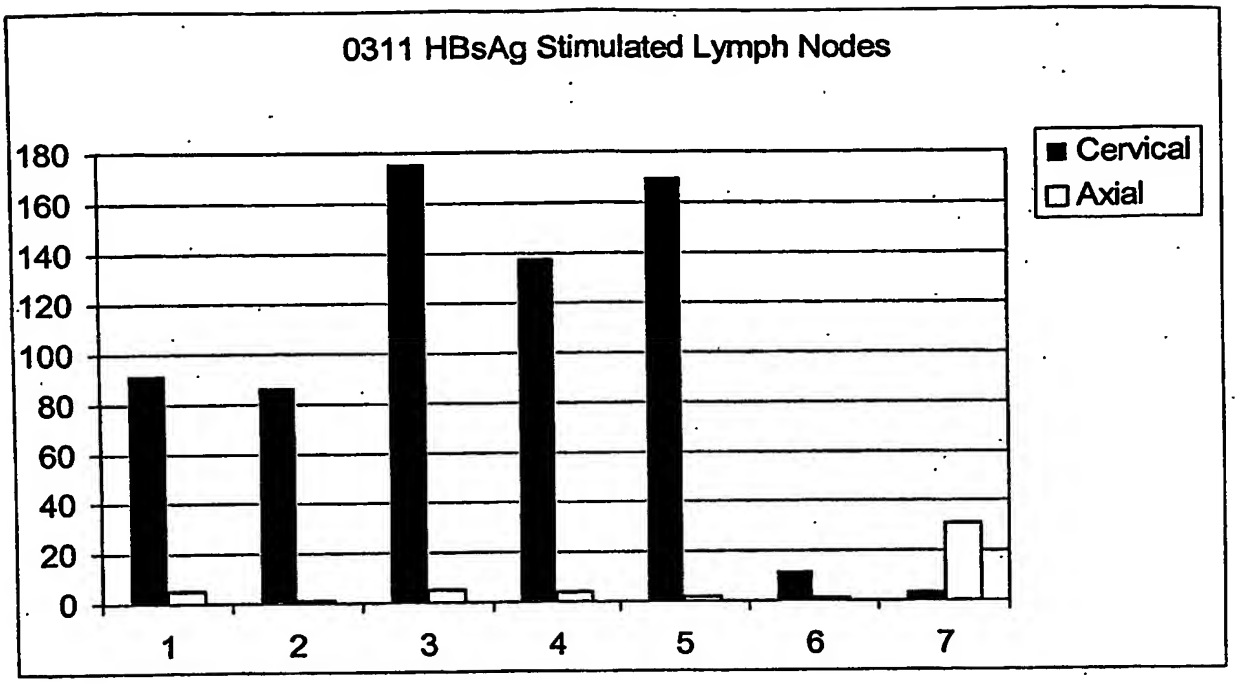


**Figure 37**
n=pooled lymph nodes from 3-4 animals

| PRIME | BOOST |
|---|---|
| 1. DNA.HBs i.m. | Engerix s.c. |
| 2. HBsAg + pSG2.HBs mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d. | HBsAg + MVA.HBs mix i.d |
| 4. DNA.HBs i.m. & HBsAg i.d. | HBsAg + MVA.HBs mix i.d. |
| 5. HBsAg + MVA.HBs mix i.d | HBsAg + MVA.HBs mix i.d |
| 6. DNA.HBs i.m. | MVA.HBs i.d |
| 7. Engerix-B s.c. | Engerix-B s.c. |

## 5.3 T-cell Responses in blood

### 5.3.1 0311b blood elispot week 4

0311b was run concurrently with 0311 and will continue until week 12. Blood is taken for elisa and blood elispot weekly until sacrifice at week 12.

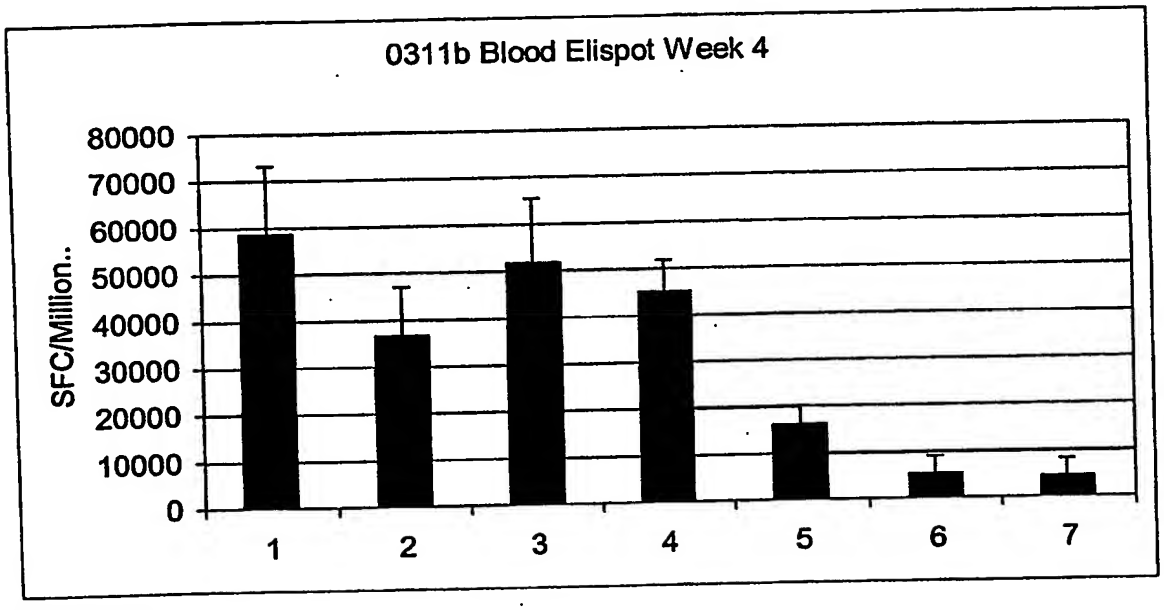


**Figure 38**
n= 4 +/- SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

Summary of responses to favourable regimes across experiments 2-5

**Antibody Responses**

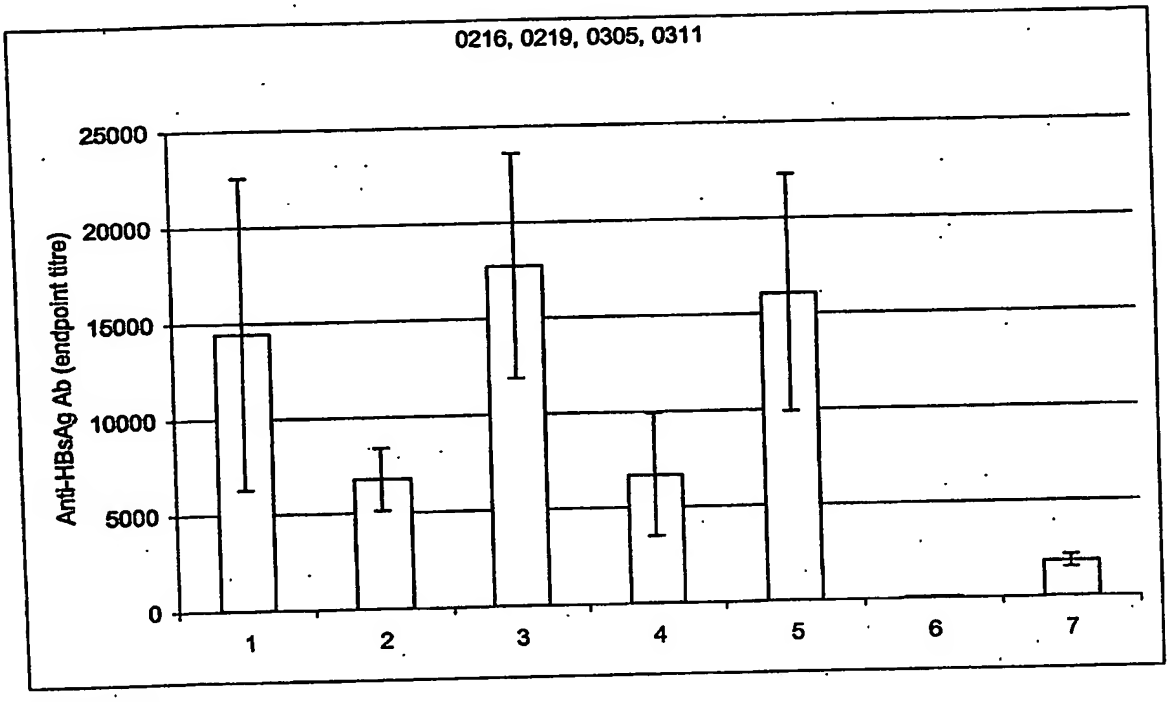


**Figure 39**
n=11-17 +/-SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

## T-cell responses

## Peptide Stimulated Splenocytes

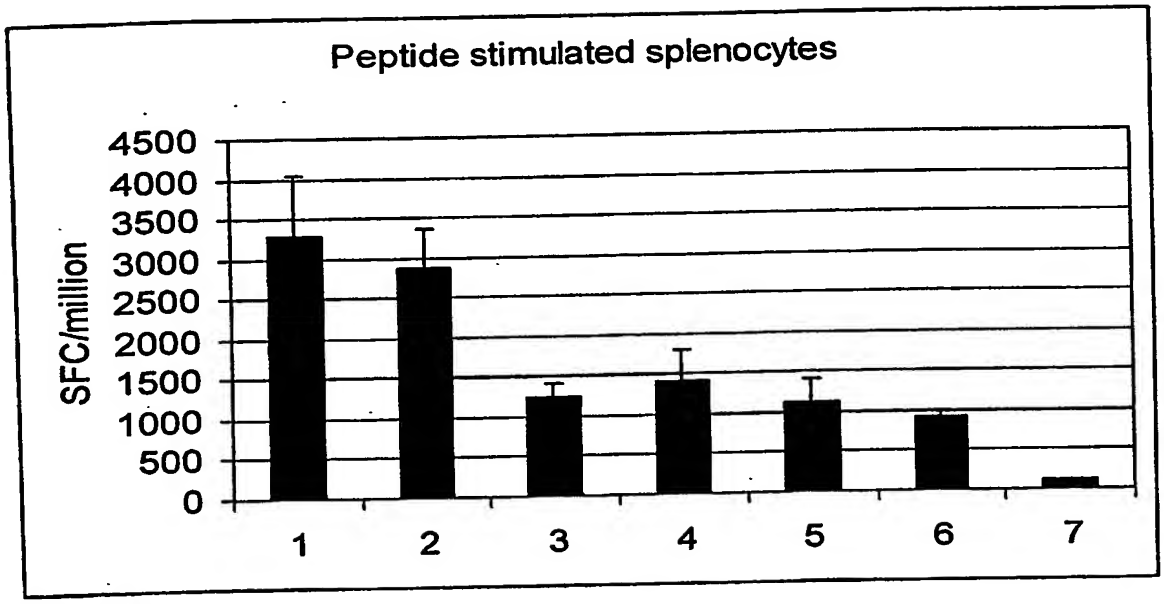


**Figure 40**
n=4-12 +/-SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

**HBsAg Stimulated Splenocytes**

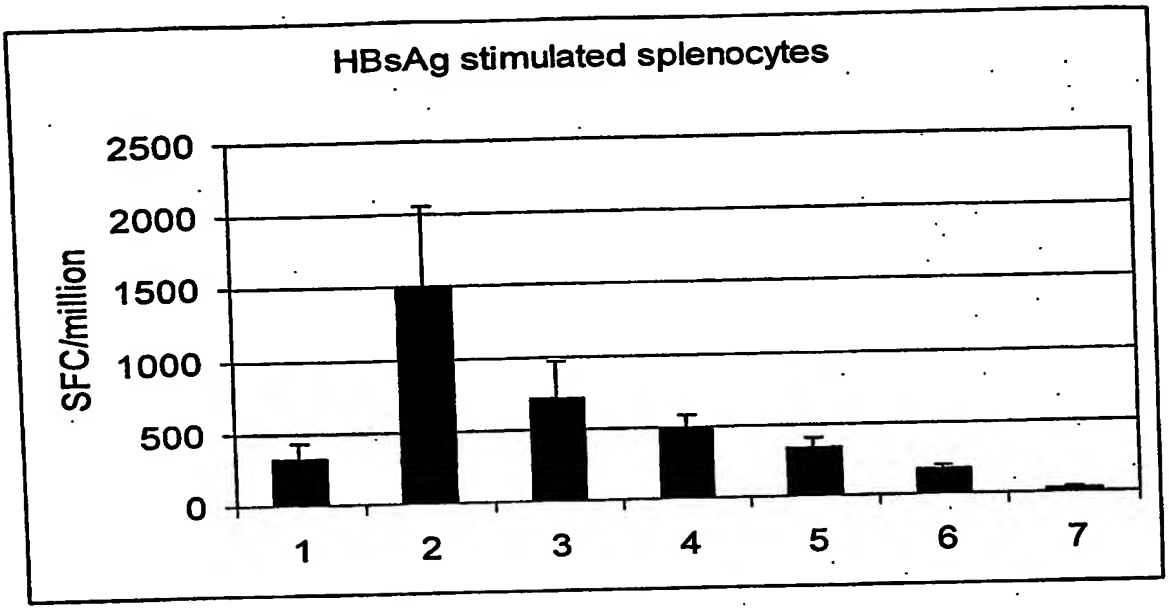


**Figure 41**
n=7-13 +/-SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

# Experiment 6

Aim; to establish whether use of Adenovirus vectors boosts antibody and cellular responses to HBsAg. Furthermmore, to establish whether heterologous immunisation induces strong cellular and humoral responses without DNA priming.

The CSP (circumsprozoite protein) from *Plasmodium berghei* (Pb)is used in some groupas the antigen, delivered using an Adenoviral vector.

## 6.1 Antibody Responses

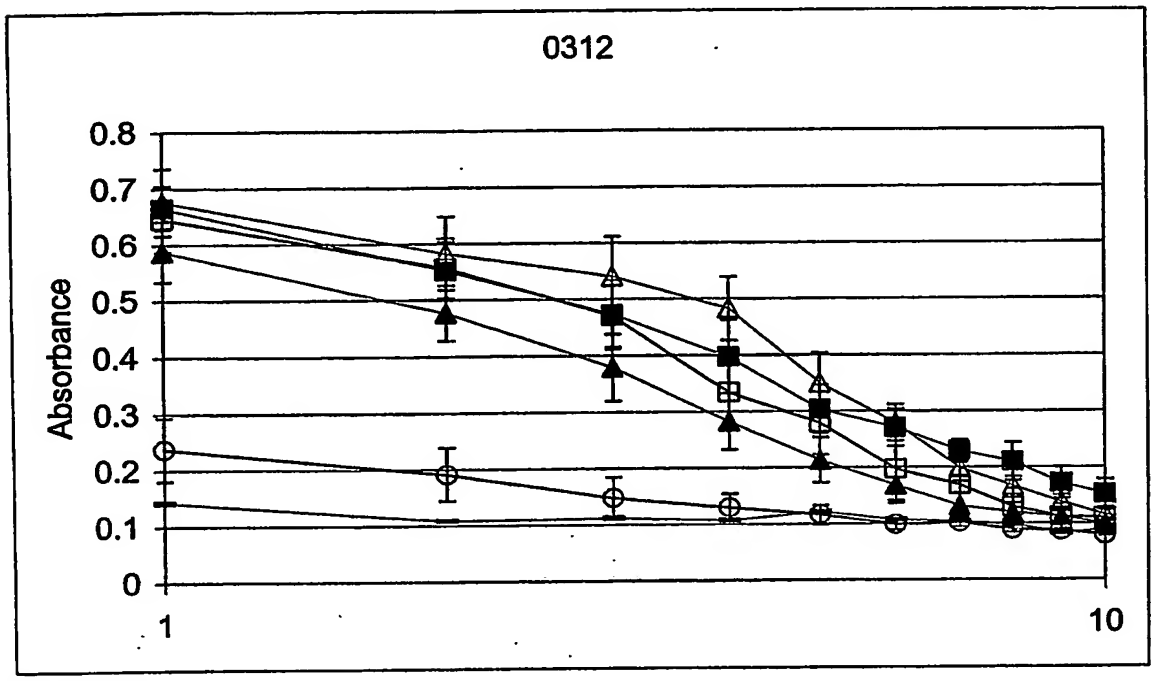


**Figure 42**

n=4 +/- SEM

| PRIME | BOOST |
|---|---|
| Δ HBsAg + FP9.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| ▲ HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| □ DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| ■ HBsAg + MVA.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| ○ DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs s.c. |
| — Naive | |

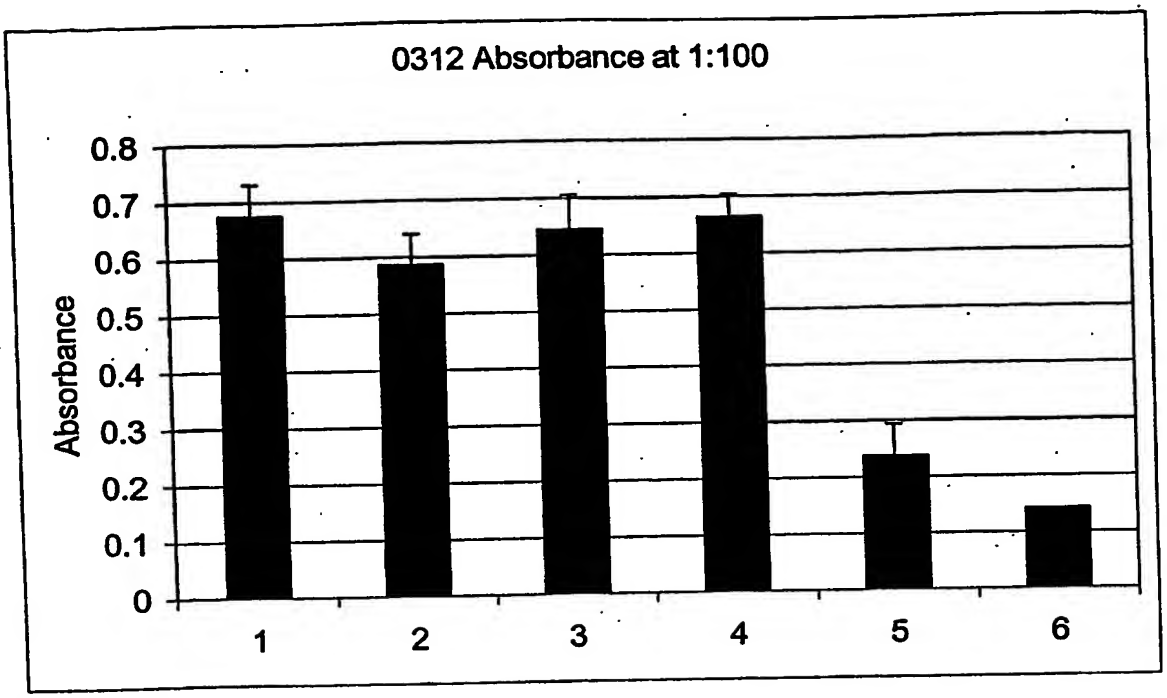


**Figure 43**

n=4 +/- SEM

PRIME

1. HBsAg + FP9.LacZ mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.
4. HBsAg + MVA.LacZ mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.
6. Naive

BOOST

HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
MVA.HBs s.c.

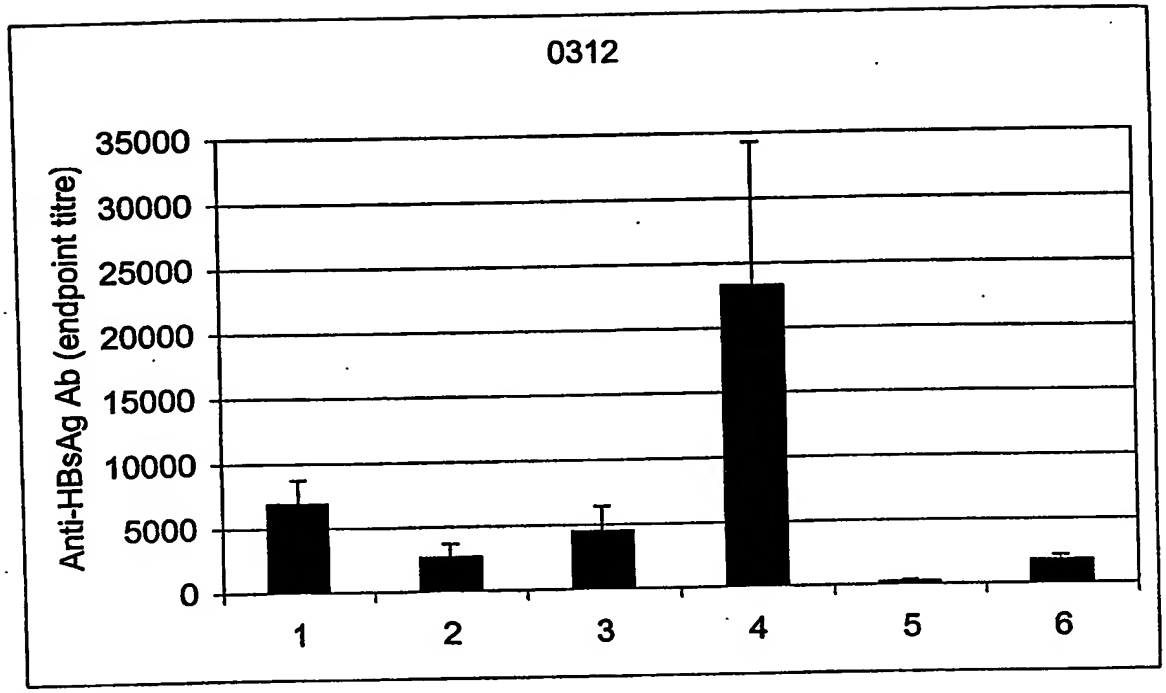


**Figure 44**

n=4 +/- SEM
(except group 6 where n=16)

PRIME

1. HBsAg + FP9.LacZ mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.
4. HBsAg + MVA.LacZ mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.
6. Engerix-B s.c.

BOOST

HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
MVA.HBs s.c.
Engerix-B s.c. (exp. 2-5)

## 6.2 T-Cell Responses

### 6.2.1 Peptide Stimulated Splenocytes

for groups 2 and 3, the CSP epitope was varied between the IPQ epitope and the Pb9 epitope

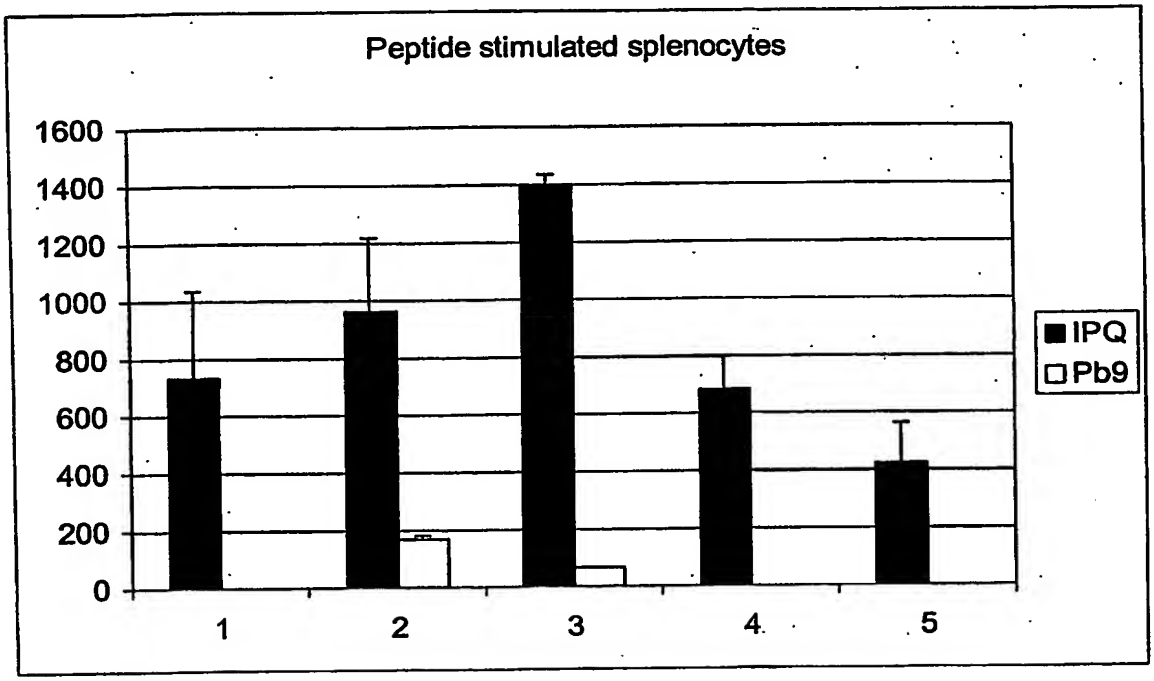


**Figure 45**

n=4 +/- SEM

| PRIME | BOOST |
|---|---|
| 1. HBsAg + FP9.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 2. HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 4. HBsAg + MVA.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 5. DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs s.c. |

## 6.2.2 HBsAg Stimulated Splenocytes

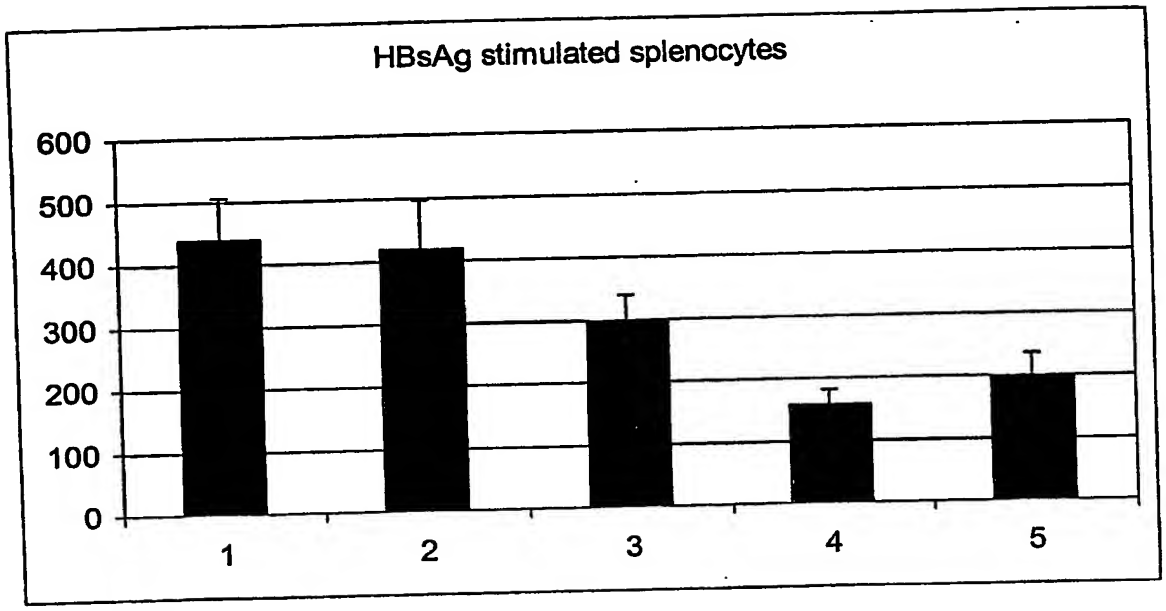


**Figure 46**

n=4 +/- SEM

PRIME

1. HBsAg + FP9.LacZ mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.
4. HBsAg + MVA.LacZ mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.

BOOST

HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
MVA.HBs s.c.

## 6.2.3 Peptide Stimulated Lymph Nodes

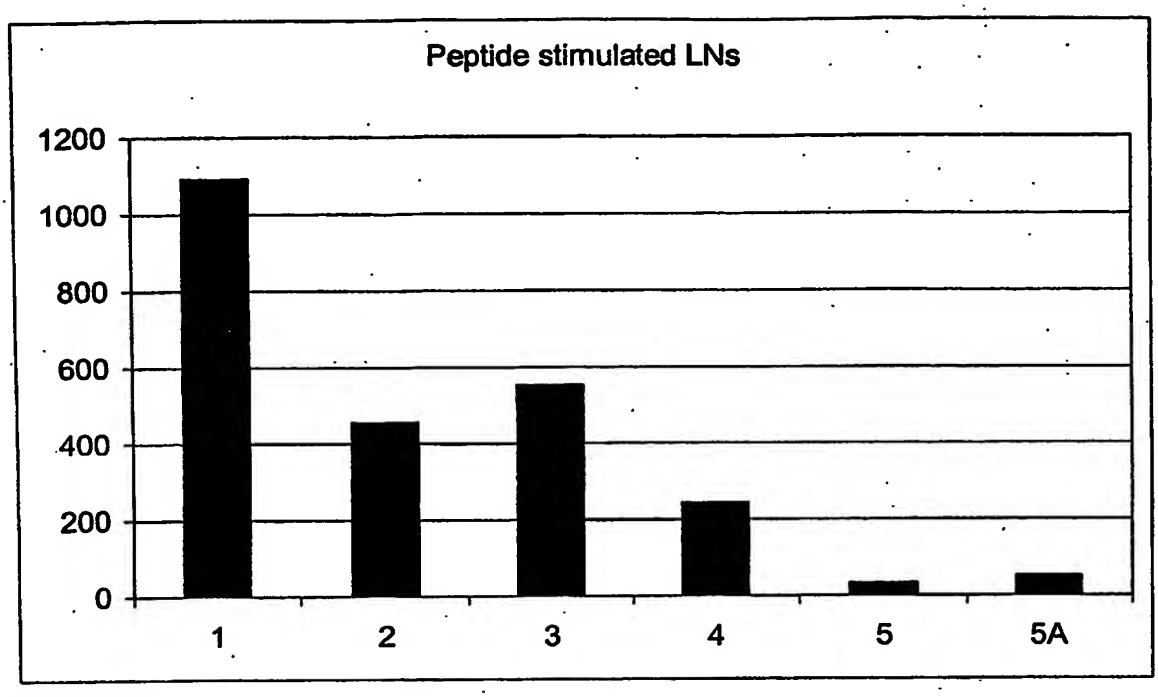


**Figure 47**

n=pooled cervical lymph nodes from 4 animals (except 5A where lymph nodes were axial)

| PRIME | BOOST |
|---|---|
| 1. HBsAg + FP9.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 2. HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 4. HBsAg + MVA.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 5. DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs s.c. |
| 5A. DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs s.c. |

## 6.2.4 HBsAg Stimulated Lymph Nodes

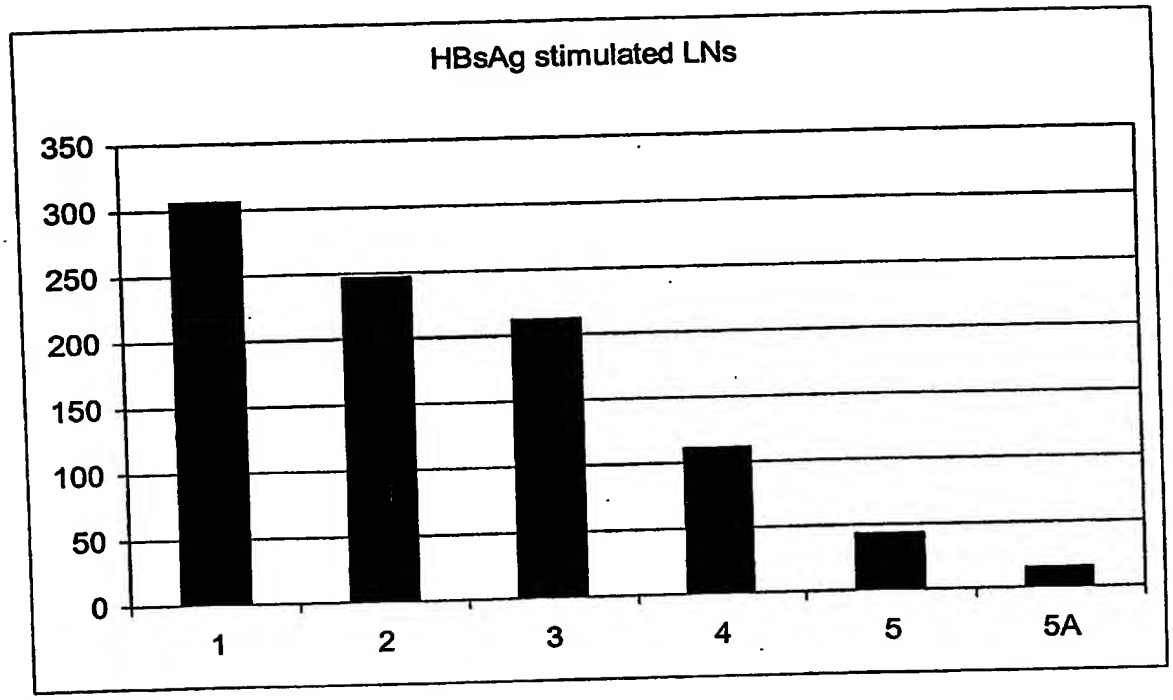


**Figure 48**

n=pooled cervical lymph nodes from 4 animals (except 5A where lymph nodes were axial)

| PRIME | BOOST |
|---|---|
| 1. HBsAg + FP9.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 2. HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 4. HBsAg + MVA.LacZ mix i.d. | HBsAg + MVA.HBs mix i.d. |
| 5. DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs s.c. |
| 5A. DNA.HBs i.m. & Engerix-B s.c. | MVA.HBs s.c. |

**CLAIMS:**

1) A vaccine, comprising a first antigen and a viral vector, formulated separately or together, wherein the antigen and the vector are formulated for co-administration.

2) A vaccine according to claim 1, wherein the antigen and the viral vector are admixed and administered as mixture.

3) A vaccine according to claim 2, wherein the viral vector is a poxvirus or an adenovirus.

4) A vaccine according to claim 3, wherein the vector is MVA or fowlpox.

5) A vaccine according to any preceding claim that induces both an effector T cell response and an antibody response, wherein the effector T cell response is not weaker than that induced by the viral vector alone, and the levels of antibody induced are not lower than those induced by administration of the antigen alone.

6) A vaccine according to any preceding claim, wherein the vector comprises nucleic acid encoding a further antigen.

7) A vaccine according to claim 6, wherein the antigen encoded by the vector is homologous to the first antigen.

8) A vaccine according to claim 7, wherein the antigen encoded by the vector is heterologous to the first antigen.

9) A vaccine according to any preceding claim, wherein the first antigen is derived from Hepatitis B, HIV, *M. tuberculosis*, *Plasmodium sp,* or is derived from a tumour.

10) A method for stimulating both humoral and antibody responses to an antigen, comprising administration of the antigen to a patient in combination with a viral vector, administration of the vector and antigen being separately or together.

# ABSTRACT

## IMPROVED VACCINES

Vaccination comprising co-administration of an antigen and a viral vector provides immunity at both humoral and antibody levels.